Historic, archived document

Do not assume content reflects current scientific knowledge, policies, or practices.

A41.9
R315An
Cop. 2

ARS 91-53

U. S. DEPT. OF AGRICULTURE
NATIONAL AGRICULTURAL LIBRARY
JUL 2
CURRENT SERIAL RECORDS

Report of

SCRAPIE SEMINAR

Held at

Washington, D.C.
January 27-30, 1964

Agricultural Research Service
U.S. DEPARTMENT OF AGRICULTURE

Trade names are used in this publication solely for the purpose of providing specific information. Mention of a trade name does not constitute a guarantee or warranty of the product by the Federal Government or an endorsement by the Government over other products not mentioned.

Numbers in parentheses refer to references placed at the end of each paper. The data in these references and those in the text are essentially those supplied by the writer of the paper.

The artwork is reproduced essentially as it was supplied by the writer of each paper.

Permission has been granted by the copyright owners for certain materials used in the following papers:

Paper No. 3, Review of Work on Scrapie at Compton, 1952-1964, by W. S. Gordon. From Journal of Comparative Pathology, Vol. 69, 1959; Vol. 70, 1960; Vol. 71, 1961; and Vol. 72, 1962; Veterinary Record, Vol. 76, 1964.

Paper No. 21, The Heredity Mechanism in the Causation of Natural Scrapie in Sheep. IV. The Possible Relationships of the Transmissible Scrapie Agent (Provirus) to the Genetical Component, by H. B. Parry. From Heredity, Vol. 17, 1962.

Paper No. 22, Review of the Avian Leukosis Complex, by B. R. Burmester, Poultry Science, 1942.

The opinions expressed at this seminar are those of the authors and publication herein does not necessarily constitute an endorsement by the Department.

Issued May 1966

PREFACE

Scrapie is a chronic, fatal, wasting disease of sheep and goats affecting their central nervous system causing them to rub and scratch. It is characterized by a long incubation period, nervousness, and loss of condition.

A scrapie seminar was sponsored by the Animal Disease Eradication Division, Agricultural Research Service, U.S. Department of Agriculture, Washington, D.C., for the purpose of reviewing and discussing all aspects of the disease.

At this seminar, world authorities on scrapie and research scientists from England, Scotland, Canada, and the United States presented their research findings concerning the causative agent, method of transmission, animal susceptibility or resistance, and the economic importance of the disease. Their papers and the discussions that followed are included in this publication.

CONTENTS

Page

List of participants and guests v

I. Introduction and purpose of the meeting
E. R. Goode, Jr. 1
R. J. Anderson 1

II. Scientific presentations and discussions
Paper No. 1--The phony disease of peach, L. C. Cochran 3
Paper No. 2--Transmission of scrapie and evidence of spread of infection in sheep at pasture, W. S. Gordon 8
Paper No. 3--Review of work on scrapie at Compton, England, 1952-1964, W. S. Gordon 19
Paper No. 4--The diagnosis of scrapie, H. A. McDaniel and L. G. Morehouse (presented by H. A. McDaniel) 41
Paper No. 5--Variation in susceptibility of sheep to scrapie and genetic implications, W. S. Gordon 53
Paper No. 6--Long-term cell cultures from the brain of a sheep affected with scrapie, D. P. Gustafson and C. L. Kanitz (presented by D. P. Gustafson) 69
Paper No. 7--Scrapie, natural and artificial: The nature of the problem, H. B. Parry 93
Paper No. 8--Natural (spontaneous) scrapie in sheep. I. Clinical manifestation and general incidence, treatment and related syndromes, H. B. Parry 95
Paper No. 9--Natural (spontaneous) scrapie in sheep. II. The pathology of natural scrapie in sheep with particular reference to the neuromuscular system, Elisabeth Beck, P. M. Daniel, and H. B. Parry (presented by H. B. Parry) 108
Paper No. 10--Natural scrapie of sheep: Its occurrence and dissemination in the field. I. The nature and relevance of evidence, H. B. Parry 117
Paper No. 11--Natural scrapie of sheep: Its occurrence and dissemination in the field. II. The general pattern of occurrence in parts of Great Britain, H. B. Parry 120
Paper No. 12--Natural scrapie in sheep: Its occurrence and dissemination in the field. III. The effect of the introduction of scrapie affected sheep into flocks and premises free of the disease, and the question of residual contamination, H. B. Parry 125
Paper No. 13--The Hereditary mechanism in the causation of natural scrapie in sheep. I. The data required to disclose hereditary influences in animal disease and its biometrical treatment, G. J. Draper 136
Paper No. 14--The hereditary mechanism in the causation of natural scrapie in sheep. III. The recessive gene hypothesis and its quantitative application to field data and statistics, G. J. Draper 139

Page

Paper No. 15--The hereditary mechanism in the causation of natural scrapie in sheep. IV. The recessive gene and apparent epidemics of contagion, G. J. Draper 146
Paper No. 16--Chronic infection of mammalian cells with VEE Virus, Henry J. Hearn, Jr..... 148
Paper No. 17--The hereditary mechanism in the causation of natural scrapie in sheep. II. Observations on one breeding group over 10 years, H. B. Parry 153
Paper No. 18--Artificial (post-inoculation) scrapie: I. The selection of sheep for experimental studies, H. B. Parry 160
Paper No. 19--Artificial (post-inoculation) scrapie: II. The response of sheep to intracerebral inoculation, H. B. Parry 162
Paper No. 20--Artificial (post-inoculation) scrapie. III. A preliminary report on artificially transmitted scrapie in the sheep and mouse, A. Vince, (presented by H. B. Parry)..... 166
Paper No. 21--The hereditary mechanism in the causation of natural scrapie in sheep. IV. The possible relationships of the transmissible scrapie agent (provirus) to the genetical component, H. B. Parry 168
Paper No. 22--Review of the avian leukosis complex, B. R. Burmester. 171
Paper No. 23--Scrapie, a review of the problem, J. T. Stamp..... 187
Paper No. 24--Field observations on scrapie incidence, G. B. Young, J. T. Stamp, C. C. Renwick, and A. G. Dickinson (presented by A. G. Dickinson)..... 199
Paper No. 25--The distribution of scrapie in sheep of different ages, A. G. Dickinson, G. B. Young, J. T. Stamp, and C. C. Renwick (presented by A. G. Dickinson)..... 207
Paper No. 26--The pathology of scrapie, I. Zlotnick (presented by J. T. Stamp)..... 213
Paper No. 27--A "scrapie"-like disease of mink, D. Burger 225
Paper No. 28--Scrapie in Suffolk sheep: A genetical investigation using experimental matings, A. G. Dickinson, G. B. Young, J. T. Stamp, and C. C. Renwick (presented by A. G. Dickinson)..... 228
Paper No. 29--Scrapie: Experiments involving maternal transmission in sheep, A. G. Dickinson, G. B. Young, and C. C. Renwick (presented by A. G. Dickinson)..... 244
Paper No. 30--Genetical control of susceptibility to experimental challenge with scrapie in Cheviot sheep, A. G. Dickinson, J. T. Stamp, C. C. Renwick, and W. Smith (presented by A. G. Dickinson)..... 249
Paper No. 31--Adaptation of the SSBP/1 scrapie agent from sheep to mice, A. G. Dickinson and W. Smith (presented by A. G. Dickinson)..... 251
Paper No. 32--Genetical control of the incubation period in mice of the neurological disease, scrapie, A. G. Dickinson and J. M. K. Mackay (presented by A. G. Dickinson)..... 253
Paper No. 33--Transmission by contact of scrapie in mice, A. G. Dickinson, J. M. K. Mackay, and I. Zlotnick (presented by A. G. Dickinson)..... 259

Page

Paper No. 34--The transmission of scrapie to mice and hamsters, I. Zlotnick (presented by J. T. Stamp) 263
Paper No. 35--An investigation into the nature of the causal agent of sheep scrapie and some observations on the biochemistry of the disease, D. L. Mould and J. S. Slater (presented by J. T. Stamp) 270
Paper No. 36--Tissue culture studies on scrapie, J. G. Brotherston, J. T. Vantsis, J. M. K. Mackay, and A. Drysdale (presented by J. T. Stamp) 282
Paper No. 37--Evolution of scrapie virus infection in mice, C. M. Eklund, R. C. Kennedy, and W. J. Hadlow (presented by W. J. Hadlow) 288
Paper No. 38--Further observations in mice following inoculation of scrapie goat and sheep brain material, C. J. Gibbs, D. C. Gadjdusek, and J. A. Morris (presented by C. J. Gibbs and J. A. Morris) 292
_____--A Review of scrapie program in the United States, R. J. Anderson 299
Paper No. 39--Certain biological situations in the animal and plant kingdoms possibly relevant to scrapie, H. B. Parry 300
Paper No. 40--The control and possible eradication of natural scrapie in sheep. I. The definition and diagnosis, H. B. Parry... 303

III. Presentations and statements relevant to the control and eradication of scrapie, as presented in conjunction with the seminar

Paper No. 41--The control and possible eradication of natural scrapie in sheep. II. The control of disorders in which hereditary factors are of paramount practical importance: Some results with scrapie in Great Britain, 1954-1963, H. B. Parry 310
Paper No. 42--The control and possible eradication of natural scrapie in sheep. III. The relationship of scrapie to the livestock industry, H. B. Parry 314
Paper No. 43--The control and possible eradication of natural scrapie in sheep. IV. The practical problems of flockmasters, T. W. Steele 316
Paper No. 44--The control and possible eradication of natural scrapie in sheep. V. A realistic programme for the containment of scrapie, H. B. Parry 322
Paper No. 45--Scrapie in the United States and the Scrapie Eradication Program, J. L. Hourrigan 340
Paper No. 46--Scrapie, its control and eradication in Canada, (statement by K. G. Wells) 361
Paper No. 47--The scrapie problem in the United States, (statement by T. C. King) 367
Paper No. 48--The scrapie problem in the United States, (statement by P. L. Linn) 368

LIST OF PARTICIPANTS AND GUESTS

R. J. ANDERSON, D. V.M.
Chairman of the Scrapie Program Evaluation Group, Deputy Administrator, Agricultural Research Service, United States Department of Agriculture, Wash., D.C.

W. L. BENDIX, D.V.M.
State Veterinarian, Richmond, Va.

L. G. BERG, D.V.M.
Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture, Charleston, W. Va.

W. B. BOUCHER, D.V.M.
School of Veterinary Medicine, University of Pennsylvania, New Bolton Center, Kennett Square, Pa.

DIETER BURGER, M.S., D.V.M.
Microbiologist, National Institutes of Health, United States Department of Health, Education and Welfare, University of Wisconsin, Madison, Wis.

B. R. BURMESTER, B.S., M.A., D.V.M., Ph.D.
Animal Husbandry Division, Agricultural Research Service, United States Department of Agriculture, East Lansing, Mich.

R. C. CARTER, Ph.D.
Virginia Polytechnic Institute, Blacksburg, Va.

L. C. COCHRAN, B.S.A., M.S., Ph.D.
Crops Research Division, Fruit and Nuts Crop Research Branch, United States Department of Agriculture, Beltsville, Md.

A. G. DICKINSON, B.Sc., Ph.D.
Animal Breeding Research Organization, Agricultural Research Council, Edinburgh, Scotland.

G. J. DRAPER
Unit of Biometry, University of Oxford, Oxford, England.

C. M. EKLUND, M.D.
National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Public Health Service, United States Department of Health, Education and Welfare, Hamilton, Mont.

STANLEY R. GATES
American Cheviot Sheep Society, Inc., Lafayette Hill, Pa.

C. J. GIBBS, JR., A.B., M.S., Ph.D.
National Institute for Neurological Diseases and Blindness, National Institutes of Health, United States Department of Health, Education and Welfare, Bethesda, Md.

R. A. GILMAN
Secretary of the American Hampshire Sheep Association, Stuart, Iowa.

EDWIN R. GOODE, D.V.M.
Chairman of Seminar, Assistant to the Administrator, Agricultural Research Service, United States Department of Agriculture, Wash., D.C.

W. S. GORDON, C.B.E., Ph.D., M.R.C.V.S., F.R.S.E.
Director, Agricultural Research Council, Institutes for Research on Animal Diseases, Compton, England.

D. P. GUSTAFSON, D.V.M., Ph.D.
School of Veterinary Science and Medicine, Purdue University, Lafayette, Ind.

W. J. HADLOW, D.V.M.
National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Public Health Service, United States Department of Health, Education and Welfare, Hamilton, Mont.

H. J. HEARN, B.A., M.A., Ph.D.
Virus and Rickettsiae Division, United States Army Biological Laboratories, Fort Detrick, Frederick, Md.

JOHN HOTCHIN, M.D.
Division of Laboratory and Research, New York Department of Health, Albany, N.Y.

J. L. HOURRIGAN, D.V.M.
Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture, Hyattsville, Md.

F. B. HUTT, Ph. D., D.Sc.
Professor of Animal Genetics, New York State College of Agriculture, Cornell University, Ithaca, N.Y.

SHERWOOD JACKSON
Illinois Suffolk Breeder, Seneca, Ill.

RUE JENSEN, D.V.M., Ph.D.
Dean, College of Veterinary Medicine, Colorado State University, Fort Collins, Colo.

H. W. JOHNSON, D.V.M.
Director, Animal Disease and Parasite Research Division, Agricultural Research Service, United States Department of Agriculture, Beltsville, Md.

T. A. KINCAID
National Wool Growers Association, La Vernia, Tex.

T. L. KING, Ph.D.
Director, American Cheviot Sheep Society, White House Station, N.J.

L. T. KURLAND, M.D., D.PH.
National Institutes of Health, United States Department of Health, Education and Welfare, Bethesda, Md.

P. L. LINN
President, National Suffolk Sheep Association, Bucyrus, Ohio.

B. H. MAYALL, M.D.
Department of Radiology, University of Pennsylvania, Philadelphia, Pa.

H. A. MCDANIEL, D.V.M.
National Animal Disease Laboratory, Agricultural Research Service, United States Department of Agriculture, Ames, Iowa.

C. J. MIKEL, D.V.M.
Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture, Atlanta, Ga.

J. A. MORRIS, B.S., M.S., Ph.D.
Division of Biological Standards, National Institutes of Health, United States Department of Health, Education and Welfare, Bethesda, Md.

F. J. MULHERN, D.V.M.
Director, Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture, Hyattsville, Md.

H. B. PARRY, M.A.Camb., M.R.C.V.S.
Nuffield Institute for Medical Research, University of Oxford, Oxford, England.

P.J.S. PLUMMER, D.V.M.
Canadian Department of Agriculture, Quebec, Canada.

TED REA, D.V.M.
Animal Disease Eradication Division, Agricultural Research Service, United States Department of Agriculture, Austin, Tex.

L. A. ROSNER, D.V.M.
President, United States Livestock Sanitary Association; State Veterinarian, Missouri, Jefferson City, Mo.

J. T. STAMP, D.Sc., M.R.C.V.S., F.R.S.E.
Director, Moredun Institute, Gilmerton, Edinburgh, Scotland.

T. W. STEELE
Flockmaster, Elmley Castle, Netherton, Worcestershire, England.

J. E. STUART, D.V.M.
Past Chief, Division of Animal Industry, State Department of Agriculture, Los Osos, Calif.

P. W. SWISHER
Commissioner of Colorado State Department of Agriculture, Denver, Colo.

O. H. TIMM
Chairman, Health Committee of the California Wool Growers Association, Dixon, Calif.

S. B. WALKER, D.V.M.
Texas Animal Health Commission, Austin, Tex.

E. E. WEDMAN, D.V.M., Ph.D.
National Animal Disease Laboratory, Agricultural Research Service, United States Department of Agriculture, Ames, Iowa.

K. F. WELLS, D.V.M., M.R.S.H.
Veterinary Director General, Health of Animals Branch, Department of Agriculture, Ottawa, Ontario, Canada.

I. INTRODUCTION AND PURPOSE OF THE MEETING

E. R. Goode and R. J. Anderson[1]

Dr. Goode:

The purpose of this seminar is to provide a setting conducive to the bringing forth of a full scientific evaluation of our present knowledge on scrapie.

Scrapie has been known to plague sheep for some 200 years or more and the complex and somewhat perplexing nature of this disease has thwarted the many attempts to fully understand its nature.

Recent years have seen more concerted efforts by a number of able investigators making highly sophisticated scientific explorations into the different aspects of scrapie. We have some of these scientists with us today and are relying upon them for a full discussion of their research--their findings and views on this subject--in order that our judgments can be based on the best and most recent information available.

Scrapie has long been a real problem to the veterinary profession and the sheep and wool producing industry. Today, more than ever, it is of academic importance to the biological and medical sciences in general. A full and proven understanding of scrapie will undoubtedly open new frontiers for exploration in a number of equally perplexing conditions associated with research being conducted on the genetics, mechanisms of resistance and susceptibility, population dynamics, and influence of environment on species of both the animal and plant kingdoms.

Accordingly, research not directly oriented to any of the multiple facets of scrapie offers promise of providing new concepts valuable to the understanding of the problem. To this end, there are included in our agenda, presentations by scientists who have researched problems that are distinctly different in most respects, but similar to scrapie in some others.

It is my sincere desire that maximum advantage be derived from this seminar by having each and every topic presented and fully discussed. The time allotted each topic, as it appears on the agenda, is the time requested by each author and I must appeal to each speaker to stay within the time limit, so that complete coverage and discussion may be accomplished.

An open meeting has been scheduled to follow this seminar, and at that time there will be discussions on the relationship of our knowledge of scrapie to husbandry and regulatory program practices. For this reason, I feel that maximum benefits will be derived from this seminar if our comments and questions are limited to the scientific aspects of scrapie.

I know that all of us hope to see the day when the many questions and differences in opinion regarding scrapie have been resolved. To think that this might be accomplished at this seminar is a very noble thought, indeed. It is realistic, however, to believe that this meeting allows a unique opportunity, with so many of us having a variety of interests and experience, to hear of the advances in knowledge and the contributions which have been made by our colleagues.

Dr. Anderson:

Most of you know, but for those that do not, the Department is presently conducting a review of the Cooperative Scrapie Eradication

[1] Chairman of Seminar and Assistant to the Administrator, ARS, USDA, and Chairman of the Scrapie Program Evaluation Group and Deputy Administrator, ARS, USDA, Washington, D.C., respectively.

Program; and as a means of bringing together all of the scientific and field experience that is known about scrapie they arranged for this scientific seminar.

We have also selected a group of breeders, regulatory officials, and research workers knowledgeable in scrapie and its effect on the sheep industry of this country to serve as consultants--as an evaluation group. They are in attendance now at this seminar. We have planned a meeting of this evaluation group for Thursday and Friday which will be entirely separate and apart from the research seminar.

The first part of that meeting, which will be Thursday afternoon, will be an open meeting in which any or all of you will be welcome to attend and participate in the discussions. The open meeting will give the evaluation group members an opportunity to question research workers and others about technical aspects of the disease that will be useful to them in making their evaluation.

Friday will be a closed meeting which will be attended by the members of the evaluation group. The Department will fully consider the opinions and views and any recommendation that this group may make in making its final evaluation of the program to be conducted. So we certainly appreciate your being here and participating in the meetings.

Dr. Goode:

We will now move into our Scrapie Seminar program.

II. SCIENTIFIC PRESENTATION AND DISCUSSIONS

Paper No. 1

THE PHONY DISEASE OF PEACH

L. C. Cochran [1]

The purpose of this talk is to describe a plant disease, the etiology of which, in the early stages of its investigation, was both confounding and obscure. To this end, the phony disease of peaches and plums is an ideal example.

How plant virus diseases are diagnosed. Before going into the details of the phony disease, I should like to briefly outline the procedure used to diagnose virus infection in plants. Since viruses are obligative parasites, they cannot be cultured on artificial media as can most bacteria and fungi. Some can exist in crystaline form in the test tube, in dried infected plant parts, or in their animal vectors (insects, mites, and nematodes); a few are transmitted by fungi. Plant viruses can reproduce only in living host plants and in some of their vectors.

When a plant abnormality, evidenced by mosaic type mottling, distorted or abnormal growth, or necrosis is suspected of being caused by a virus, attempts are made to transmit it from diseased to healthy plants of the same sort. Transmission may be effected by one of several methods--(1) mechanically by rubbing leaves of the test plant with sap or cut tissue of the diseased plant, (2) by grafting tissue from the diseased plant onto the test plant and (3) by feeding previously non-viruliferous vectors on the diseased plant and in turn on the test plants. For woody plants, the grafting method is most commonly employed. If characteristic symptoms develop on the inoculated test plants, both methods 1 and 3 are investigated. Usually the vectors are unknown and this then involves a separate investigation. Successful graft transmission, although highly indicative, is not enough to prove virus etiology. Several fungi and a few bacteria and protozoans are known which infect plants and become systemic in their host tissues and can pass graft unions, therefore cultural techniques have to be carried out to eliminate organisms other than virus. Diseases successfully transmitted by all three methods are generally considered to have virus etiology. Viruses which can be transmitted mechanically in juice can often be purified and photographed and the purified preparations then checked for biological activity.

History of Phony Disease

The first record of the phony disease was two dwarfed trees observed by Samuel Rumph, the originator of the Elberta peach, on his property at Marshallville, Georgia, about 1885. The dwarfed trees were called pony peaches because of their size and were saved as curiosities, not realizing that the disorder was later to spread and result in the loss of over two million peach trees in the Southeast. The name later became changed to phony, a term commonly used in the early twentieth century to refer to information of doubtful validity in

[1]Chief, Fruit and Nuts Crops Research Branch, Crops Research Division, ARS, USDA, Beltsville, Md.

keeping with the lack of faith in the early stages of the development of the telephone. The number of phony trees increased steadily until by 1915, thousands were affected and growers asked for help. Research was begun in 1921 by the then Bureau of Plant Industry of the U.S. Department of Agriculture, and in 1928 the etiology was established to be definitely an infective virus. The disease continued to spread, reaching a peak in the early 1940's at which time it had been found in 13 southeastern States. Factual information developed through research has provided a basis for control until at the present time, the annual loss from phony has been reduced to a fraction of one percent.

Why the Disease was Baffling

When the phony disease was first called to the attention of research personnel, it was regarded as an oddity resembling a genetic disorder more than an infectious disease. Affected trees were not unfruitful, the fruits were not off-flavor, there was no dieback or death of trees. In fact, the affected trees were darker green and appeared to have more leaves and be more healthy than normal but their fruits were small and the yield progressively declined annually. Actually, affected trees had less leaves but appeared more lush because the internodes were shortened bringing the leaves closer together.

There was no precedent for such a disease in plants. There were no lesions from which to isolate and the virus diseases of peach known at that time were killers with clear-cut characteristic symptoms. The continued alarming spread of phony indicated a biological cause and suggested either an insect toxin of a spreading insect or an infectious agent, one of which could be a virus.

Sequence of Investigation

Research on the phony disease was initiated in 1921 when Dr. Lee M. Hutchins was assigned to the investigation. He made careful surveys and studies of the spread pattern as well as studies of affected trees and decided that the most plausible cause was an infectious virus. He initiated large-scale transmission studies but the usual techniques where buds or scions from diseased trees were grafted onto healthy trees, failed to reproduce the disease. Where such propagating material was forced into growth, it was normal in every respect. It was indeed baffling that the mere transfer of diseased shoots to new root systems resulted in complete recovery yet the disorder continued to spread in orchards at an alarming rate.

Fortunately, in Dr. Hutchins' thoroughness, he also made combination grafts using roots from healthy trees and scions from diseased trees and conversely roots from diseased trees and scions from healthy trees. In every case where roots from diseased trees were used, the disease was reproduced. This showed that the reproducible principle was in the roots but not in the tops. This information was supplemented by a sequence of experiments which further substantiated the limitation of the reproducible principle in the roots of diseased trees, some of which are as follows:

(1) When healthy trees in pails were grafted through holes in the bottom of the pail to terminals of a diseased tree, they became phony in appearance but recovered as soon as they were severed from the diseased tree and set in a nursery row.

(2) When healthy nursery trees, set in the ground, were inarched into scaffold limbs of a phony tree and the limbs severed from the mother phony tree so as to transpose them to a new root system, the formerly diseased tops recovered.

(3) When roots and stem pieces of phony trees were grafted into the tops of healthy nursery test trees, the test trees receiving root pieces became phony, whereas those receiving stem pieces did not.

This information stimulated a comprehensive search for an insect vector, almost necessarily one which fed on roots but without success.

Further work with root tissue from diseased trees using bark only and bark plus wood as inoculum showed that wood was necessary in the inoculum to effect transmission. Incidental to this work, a simple chemical laboratory test was developed for aid in identification of phony. When thin sections of roots from diseased trees were dropped in acidulated methyl alcohol, pink to purple spots developed in the woody portion of the section in 1 to 2 minutes.

A reexamination of the transmission work with scions indicated that the woody portion of scions usually dies before a good union is established, even though the scion lives and grows into a tree; thus if virus had been present and was limited to the wood in the scion, it would have died before a new wood bridge between scion and stock was formed and no transmission would have taken place. A few cases of transmission were effected where phony scions were grafted onto seedling roots at the ground line from which the tops were removed without moving the roots from the soil. This work was done in the spring and undoubtedly the fact that the whole root systems of the seedlings were left in the ground undisturbed, resulted in a quick union and the virus bearing wood in the scions remained alive until union was formed. It was reasoned that phony trees resulting from diseased scions should have virus in the tops because the virus in the scions would have been carried along to new growth by cell division. Since the virus was limited to the wood of roots, it would be logical that it would be similarly limited in tops.

In the meantime, a new type of graft was developed, in which the inoculum consisted of a shield of bark subtending a short spur, termed heel-spur scion. This type of scion could be used during the growing season, the shield being inserted in a T-cut in the same manner as with budding. Since the shield was placed directly against the cambium, it resulted in a quick union and the woody portion of the spur remained alive while the union was being effected.

When heel-spur scions from phony trees which had been grown from phony scions were used as inoculum, nearly 100 percent transmission resulted. This was the first time that transmission had been effected from top to top and indicated that the infectious principle could exist in the tops of phony trees under certain conditions.

The demonstration of the infectious principle in the tops of phony trees changed the vector search to top feeding insects and raised a new horizon. It was soon found that a number of large leafhoppers, the so-called "sharp shooters," could transmit the phony disease. These insects are large ravenous feeders, which prefer woody stemmed plants; they are long lived, often living over winter. It was further found that once they acquire the phony virus, they can transmit it any time during the remainder of their life.

These findings added much to clarify the picture on the phony disease both as to its etiology and logic in its behavior and helped in the development of control. Once obscure, the phony disease now presents a logical picture and can be characterized as follows:

(1) Phony is caused by a virus which is limited to the woody cylinder of its host plants.

(2) The virus readily invades roots as contrasted to tops because there is approximately 20 times as much living tissue in the wood of roots as compared to tops but perhaps more important the wood of roots serves as storage tissue and the living portions are well interconnected as compared to tops.

(3) The virus is transmitted by a number of large leafhoppers, called "sharp shooters," in which it is persistent. These hoppers are long lived, travel long distances, and introduce the virus in the tops of trees. If they feed low in the tree, the virus moves readily to roots where it becomes uniformly distributed but it may not move upward to other parts of the top.

(4) The phony virus effects symptoms through some upset of the normal growth regulator system which controls growth elongation and fruit size. The incubation period following vector feeding varies from 18 months to 3 years and is proportional to build up of the virus in the roots.

(5) Various prunus species, including peaches, apricots, almonds, cultivated and wild plums are susceptible to phony. No cherries or cherry-like species have been infected. The main reservoir of phony outside of peach orchards is wild plums, particularly the Chickasaw plum which forms dense thickets in the Southeast.

(6) The phony virus is thermal labile; the virus is killed if infected nursery trees are immersed for 40 minutes in water maintained at 40 degrees C.

Control measures:

(1) Plant new orchards as far from old orchards as possible, preferably with 1/2 mile

isolation. Allow one year of fallow after old orchards are pulled before new orchards are planted.

(2) Remove wild plums from the immediate vicinity of orchard sites one year prior to planting.

(3) Peaches should be sprayed with good insecticides such as malathion, using a speed sprayer so as to cover orchard borders and cover crops, as well as the trees.

(4) Diseased trees should be destroyed as soon as they are recognized. A Federal-State cooperative inspection service indicates phony trees for growers, and growers furnish labor to dehorn the trees and remove the stumps.

DISCUSSION

Dr. Goode:

Thank you Dr. Cochran. Are there any questions or comments regarding Dr. Cochran's paper?

Question: What is the incubation period from the time the tree is infected until the disease appears in the tree?

Dr. Cochran:

With insect transmission, the incubation period is a minimum of 18 months, and can go up to 3 years. It depends somewhat on tree size and where the virus is put in. With grafts, where we use a healthy root, and a diseased scion, the incubation period is 18 months.

Dr. Hutt:

When you find an infected tree, do you destroy the one tree or destroy the whole orchard?

Dr. Cochran:

We destroy only the one tree.

Dr. Kurland:

Has the virus been studied by electromicroscopy, and if so, what are its characteristics?

Dr. Cochran:

No, the virus has not been studied by electromicroscopy. In order for a virus to be studied under the microscope we have to be able to isolate and purify it. To be sure that active virus is present in any such pure culture preparation we must be able to transmit it mechanically in juice. We have not been able to do that.

Mr. Yohe:

Do you know what happens to the virus during this long incubation period? Is it multiplied, is it invading cells, is there a delay--is it actually spreading through the plant with a delay in showing up of signs?

Dr. Cochran:

Yes, that has been studied quite extensively. We have put a piece of infected root, grafting it on to a small root on one side of the tree, and then have followed the sequence of spread through the root system.

We know that signs are not expressed in the top until most of the root system is invaded. By taking subcultures at various points around the tree, we know that it takes approximately 18 months for the virus to move through the root system.

Mr. Yohe:

In other words there is no dormant period here.

Dr. Cochran:

No, there is no dormant period.

Dr. Gordon:

Is there any difference in susceptibility between different strains, or lines of peaches, and is there a difference between susceptibility between peaches and the other fruits we mentioned as being susceptible to this disease?

Dr. Cochran:

No. As far as we know, all peaches, all peach varieties are equally susceptible. As far as we know this is one of our plant viruses which appears to have no strains.

There are some differences in the tissues of plants invaded. For example, in the peach, the virus is limited almost entirely to roots. Although when we grow a tree from a diseased scion, we know that the virus will be carried to all parts of the tree top as it grows by cell division.

In plums, there is apparently more living wood in the tops than in peaches, thus the virus moves more rapidly into plum tree tops.

Dr. Gustafson:

What is the relationship of the virus to the vector? How long will the virus persist in the vector? Have you made any effort for cell cultures in the vectors? Does the virus simply reside there, or does it invade the cells?

Dr. Cochran:

The vector, when placed upon a diseased tree, acquires the virus very readily. There is a lag of some 2 weeks or more before the vector can transmit the virus. But the vector retains the virus during its entire lifetime. These vectors live over winter, and we have had them live up to a year and still transmit the disease.

Dr. Mulhern:

What is the average incubation period of a plant virus?

Dr. Cochran:

That is extremely variable. We have a few plant viruses such as tobacco mosaic and cucumber mosaic which have an incubation period of a few hours up to a few days. We have at the other end of the extreme a virus disease of citrus, psorosis, which has a minimum incubation period of 6 years, an average incubation period of 10 to 12 years, and may go to 20 years before trees develop signs. In the meantime, the psorosis virus can be transmitted from infected trees that are not yet showing signs.

Dr. Stuart:

How long have you been using the system of pulling the infected trees to eradicate or control, and has the disease reappeared in these orchards where just the infected trees have been removed?

Dr. Cochran:

The control program of pulling diseased trees was initiated in 1929, and is still going on. Now, we know that this program had one major weakness. When we see a phony tree there are probably others in the vicinity in the incubation stage. Until we stopped the spread, this sort of program was not very effective. The tree removal program had to be coupled with control of the insect vector to stop the spread.

We say we should remove infected trees because the vectors might pick up virus from them. However, we now believe the bulk of the infection in an orchard comes in from the outside.

Dr. Anderson:

What about the disease in the nursery--do they permit movement of the nursery stock?

Dr. Cochran:

In general, peaches in the Southeast are propagated by a method we call June budding. The seed is sown in the spring and the seedling is large enough to bud in June. In June budding only the bark with the bud on it is used. The wood is removed and the fresh bark is placed right against the cambium to get a quicker union which will allow a saleable tree to grow in one year. In the case of June budding we get no transmission whatever. Where we use a fall bud with some wood in it, it is very rare that phony is ever transmitted because usually that chip of wood dies before there is any union. We have not been able to transmit phony that way. Phony is not a nursery disease. However, we do restrict the movement of nursery stock to within the infected area, and there is a limitation on the growing of nursery stock. It can't be grown within a quarter of a mile of an orchard that has phony in it.

Dr. Goode:

Dr. Cochran, this paper has been of great interest to this group. There have been quite a few very obscure portions of this tree disease that had to be ferreted out and recognized before some of this work could even be initiated. I think we are facing a similar situation with scrapie.

TRANSMISSION OF SCRAPIE AND EVIDENCE OF SPREAD OF INFECTION IN SHEEP AT PASTURE

W. S. Gordon[1]

In introducing the subject of scrapie disease in sheep, I make no excuse for reiterating some of the remarks I made in 1959 at the 63rd Annual Meeting of the U.S. Livestock Sanitary Association in San Francisco (1, 2, 3, 4), because I think that some of the early observations both of the experimental disease and the naturally occurring condition are of fundamental importance in focussing attention on the problems that have yet to be solved in connection with the epidemiology of this baffling disease.

A great deal of scepticism has been expressed from time to time about the transmissibility of scrapie and, had it not been for the unfortunate accidental transmission of the disease to a large number of sheep through the contamination of Louping-ill vaccine with scrapie infective material, I might also be among the ranks of the sceptics.

A stage has now been reached, however, in which it is clear that the transmissible agent can be passed in series with cell-free filtrates indefinitely through groups of sheep. It is also clear that it can be passed from sheep to goats and back to sheep, from sheep to mice, from goats to mice and back to sheep and goats and from mice to hamsters and also to rats.

Because it is filterable, the transmissible agent might be characterised as a virus, but it differs from other viruses in that it produces a progressive degenerative disorder of the central nervous system, the lesion being bilaterally symmetrical, whereas most other viruses produce an inflammatory-type reaction. Despite many attempts to demonstrate antibody response, none has been detected so far. Further, the disease has a remarkably prolonged incubation period and the agent can withstand boiling and also 10% formalin for periods up to 28 months without being inactivated.

When first I became associated with the investigation of scrapie I thought it had the appearance of being a hereditary disease and was not likely to be transmissible by inoculation. However, over the course of years several incidents occurred which convinced me that scrapie was inoculable and that, under certain circumstances, the possibility of spread from affected to healthy animals at pasture could not be excluded. This view is strengthened particularly by the writings of earlier workers, notably Sir Stewart Stockman (5), and Sir John M'Fadyean (6), from whose writing I quote:-

> "Fortunately, I had the opportunity in 1912 to be brought into touch with two owners whose flocks had been infected recently and was able to gather from them valuable information while the important facts were still fresh in their memories.
>
> The following facts were ascertained with regard to the first of the flocks, owned by Mr. A.
>
> Mr. A. had been tenant of the farm for 11 years prior to 1907, and during that period he never had a case of scrapie. Up to that time also the disease, so far as he was aware, did not exist on any of the neighbouring farms.
>
> In the autumn of 1907 Mr. A. bought 140 Cheviot ewe lambs at a public auction sale in a neighbouring town. They had been bred by a Mr. X. on a farm in another county and

[1] Director, Agricultural Research Council Field Station, Compton, Berkshire, England.

it was subsequently ascertained that scrapie existed in this stock.

These lambs were put to the rams in November, 1908, along with thirty other young ewes of the same age (so-called gimmers), which had been bred by Mr. A. on his own farm.

The total ewe stock, including these gimmers, was about 560. The flock had a very wide run of hill pasture, on which, however, there was no strict separation between any of the different lots of sheep.

In February and March of 1909, following the usual custom, the pregnant ewes were taken off the hill pasture and folded together on turnips.

The first symptoms of scrapie in the flock were seen in February, 1909, and during the next six or seven months about thirty ewes were lost from the disease. Without exception, all of the ewes attacked belonged to the lot of 140 which had been bred by Mr. X. and bought by Mr. A. in the autumn of 1907.

In the autumn of 1909, Mr. A. determined to endeavour to eradicate the disease, and with that object he fattened and sold all the surviving ewes of this lot, and at the same time he sold the whole of their lambs. Hence, after the autumn of 1909 none of the ewes bred by Mr. X. and none of the lambs out of these were left on Mr. A's. farm.

From October 1909 till April 1911 (a period of 18 months), Mr. A.'s farm appeared to be free from scrapie, but in April 1911 two ewes were attacked. Two more were attacked in the summer and several more were noticed to be affected in November.

From that time onwards cases cropped up at intervals, and at the time of my visit in August 1912 four ewes were affected.

On the second farm, tenanted by Mr. B., there was an almost identical history with regard to the origin of scrapie.

Mr. B. had also been free from the disease up to the spring of 1909, and all the cases of scrapie which occurred in his stock in that year were among gimmers which had been bought as lambs in the autumn of 1907. These lambs, like those purchased at the same time by Mr. A., had been bred by Mr. X. on a farm in another county.

Feeling greatly alarmed by the occurrence of the disease on his farm, Mr. B. determined to root it out, and in the autumn of 1909 he sold all the surviving ewes which had been bred by Mr. X. and also all their lambs. After this there was no disease on Mr. B.'s farm until the spring of 1911, when it broke out again among ewes that had been in his possession before the autumn of 1907.

The facts, which have been stated above, may be summarised by saying that in two large stocks of sheep the disease was, according to testimony that appears to be quite trustworthy, introduced by the purchase of apparently healthy lambs from an infected flock; that a notable proportion of these lambs developed the disease when they were about two years of age and that, in spite of the sale of all the animals of this lot and their progeny before the next breeding season, the disease subsequently developed in the other ewes, and in fact established itself on the farms. (Information obtained later was to the effect that there was no diminution in the number of cases during the year 1917 on the first of the two farms).

It will probably be generally conceded that if the facts were as stated above and none of importance has been omitted, they suggest that scrapie is caused by the multiplication of some parasite in the sheep's body, and is spread by the direct or indirect transference of this parasite from the diseased to the previously healthy".

This situation is similar to that described for a farm in Illinois, where the occurrence of disease in purchased stock led to the slaughter of the whole flock. This was referred to in an issue of "The Shepherd" magazine as "The skies fall on Illinois" and "Nations wonder why". As long as a programme of eradication is being pursued no alternative action was possible because if, in 18 months time, scrapie had appeared in the native stock, as in the situation described by M'Fadyean, and if this flock had become a source of infection for other farms, the storm created by the critics of the regulatory service would have made the French Revolution look like an old ladies' tea-party.

The sequence of events which influenced my personal view of spread of infection by contact was firstly my observation of an experiment started in 1932 by Dr. Russell Greig (7) at

Moredun Institute, Edinburgh, while I was a member of his staff. In this experiment Greig exposed a normal breeding flock of 6 pregnant Cheviot ewes and 20 Half-Bred gimmers from two farms on which there was no history of scrapie, to a scrapie-contaminated environment for a period of 3 years. The normal flock was placed in a field immediately adjoining a double fenced enclosure of half an acre in which three or four natural cases of scrapie were maintained. Approximately twice per week for a period of 3 years the normal flock was interchanged with the affected animals and, as far as possible the two groups were prevented from having direct contact. The normal ewes were mated in 1933 and 1934 with a Cheviot ram, the progeny of one of the original pregnant Cheviot ewes.

At the end of the 3-year period, since no case of scrapie had occurred in the normal flock or their offspring, born in 1934 and 1935, the animals were given to a farmer, who was prepared to accept them, in spite of their history. The flock then comprised 16 ewes (12 Half-Breds and 4 Cheviots) and 9 female offspring. The male lambs had been castrated and retained at the Institute. Three months after the flock had been transferred to the farmer, one of the Half-Bred ewes developed scrapie and eventually a total of 10 cases occurred - 7 Half-Bred ewes, 1 Cheviot ewe, and 2 of the female offspring (fig. 1).

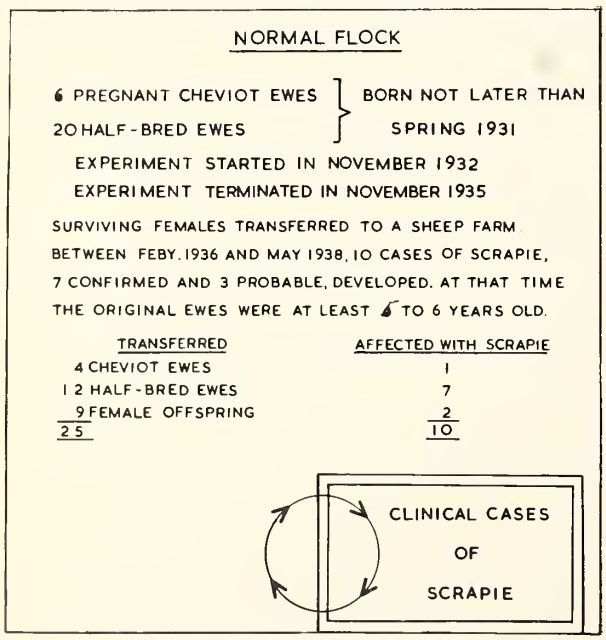

NORMAL FLOCK
6 PREGNANT CHEVIOT EWES
20 HALF-BRED EWES
BORN NOT LATER THAN SPRING 1931
EXPERIMENT STARTED IN NOVEMBER 1932
EXPERIMENT TERMINATED IN NOVEMBER 1935
SURVIVING FEMALES TRANSFERRED TO A SHEEP FARM BETWEEN FEBY. 1936 AND MAY 1938, 10 CASES OF SCRAPIE, 7 CONFIRMED AND 3 PROBABLE, DEVELOPED. AT THAT TIME THE ORIGINAL EWES WERE AT LEAST 5 TO 6 YEARS OLD.
TRANSFERRED
4 CHEVIOT EWES
12 HALF-BRED EWES
9 FEMALE OFFSPRING
25
AFFECTED WITH SCRAPIE
1
7
2
10
CLINICAL CASES OF SCRAPIE

Figure 1.

The first case developed 3 years and 3 months and the last case 5 years and 2 months after the commencement of the experiment.

It is important to examine closely the age of these animals at the time they developed scrapie:-

Case	Symptoms When Aged		Death or Destruction When Aged		Histology	
	Years	Months	Years	Months		
1	5	10	5	11	+	Half Bred Ewes
2	6	2	6	4	+	
3*	1	3	1	4	+	
4	6	5	7	7	+	
5	Not known		6	11	+	
6	6	11	7	1	?	
7	6	11	7	1	+	
8*	2	3	2	7	+	
9	7	7	7	8	?	
10	7	9	8	1	+	Cheviot

*Born 1935. +Clinical signs of scrapie confirmed histologically. ? Acceptable clinical signs not confirmed histologically.

Parry has suggested that 90 percent of sheep that develop scrapie do so by the age of $4\frac{1}{2}$ years and the remaining 10 percent are classified as late manifestations. By this reckoning it seems a peculiar coincidence that every one of the original sheep that developed scrapie in Greig's exposed flock was a late manifestation and it is difficult to find any explanation for this occurrence other than infection of the normal flock at pasture at a later age than natural cases normally occur.

Apart from field evidence, this was the first experimental warning that the disease could spread by contact and 28 years later I checked that the two farms of origin of Greig's normal flock had continued free of scrapie.

Secondly, in 1934, I was completing work at Moredun Institute on the investigation of Louping-ill, which showed it to be an encephalomyelitis of sheep, caused by a filterable virus transmitted by the tick, Ixodes ricinus (an Arborvirus). For 4 years--1931 to 1934--I had carried out field trials to test the efficiency of a vaccine for the prevention of Louping-ill, in which half of the sheep on affected farms were vaccinated and the other half left as controls. Many thousands of sheep were involved in these trials, which proved that the vaccine was safe and effective since it caused no reaction in inoculated sheep and it reduced the Louping-ill mortality from about 10 to about 1 percent. This vaccine was inoculated subcutaneously and consisted of a 10 percent suspension in saline of the brain, spinal cord and spleen harvested from sheep which, 5 days previously, had been infected experimentally with the virus of Louping-ill. The vaccine contained 0.35 percent of formalin which inactivated the Louping-ill virus. At the end of 1934 and the beginning of 1935 a considerable amount of this vaccine was made for issue on a commercial basis. The various batches of vaccine were blended into three main batches labelled 1, 2, and 3 and they were used to inoculate sheep in March and April 1935. Fifteen months after vaccination complaints about the occurrence of scrapie among vaccinated sheep began to be received.

It will be seen from figure 2 that the Batch 2 vaccine contained a factor, capable of producing scrapie, which was not present in Batches 1 and 3. Bearing in mind the long interval that had elapsed since vaccination and that the vaccine contained 0.35 percent of formalin and had passed the usual safety and sterility tests, I found it difficult to believe that these cases of scrapie were associated with vaccination. On further enquiry among the users of the vaccine, however, it became clear that Batch 2 was contaminated with an agent that was causing scrapie and that on farms where it had been used scrapie was occurring in varying incidence from a fraction percent to 30 percent. On tracing the origin of the animals used to make the 3 batches of vaccine, it was found that of 114 sheep used to make Batch 2, 8 were castrated male lambs born in 1934 in the normal flock which Dr. Greig had maintained in a scrapie environment. Since two ewe lambs, which were fellows to the eight included in the vaccine, died of scrapie respectively in 1936 and 1937, it is probable that one or more of the eight, although apparently quite normal when used in 1935, were in the incubative stage of a scrapie infection. This convinced me in no uncertain fashion that scrapie was an inoculable disease.

Thirdly, in 1938 I set up an experiment to confirm that the disease was transmissible and the results have a distinct bearing on the question of natural spread of the disease in sheep at pasture.

For this purpose the whole of the Cheviot sheep stock on a hill farm in Eskdalemuir, Selkirkshire (in the south of Scotland) was taken over. This sheep stock was well known to me because it had previously been used for experiments in the prevention of lamb dysentery and scrapie had never been known to occur on this farm. When the experiment terminated it was agreed that the surviving sheep should be slaughtered and the land planted with trees by the Forestry Commission.

It was my intention to detail this experiment as an appendix to this paper but the full analysis has not been completed in time and the result will be published elsewhere. Figure 3 will suffice to illustrate the point I wish to make.

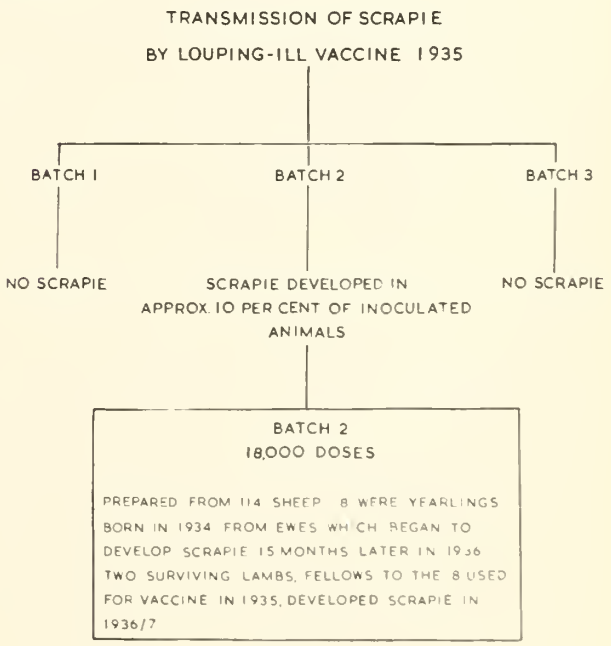
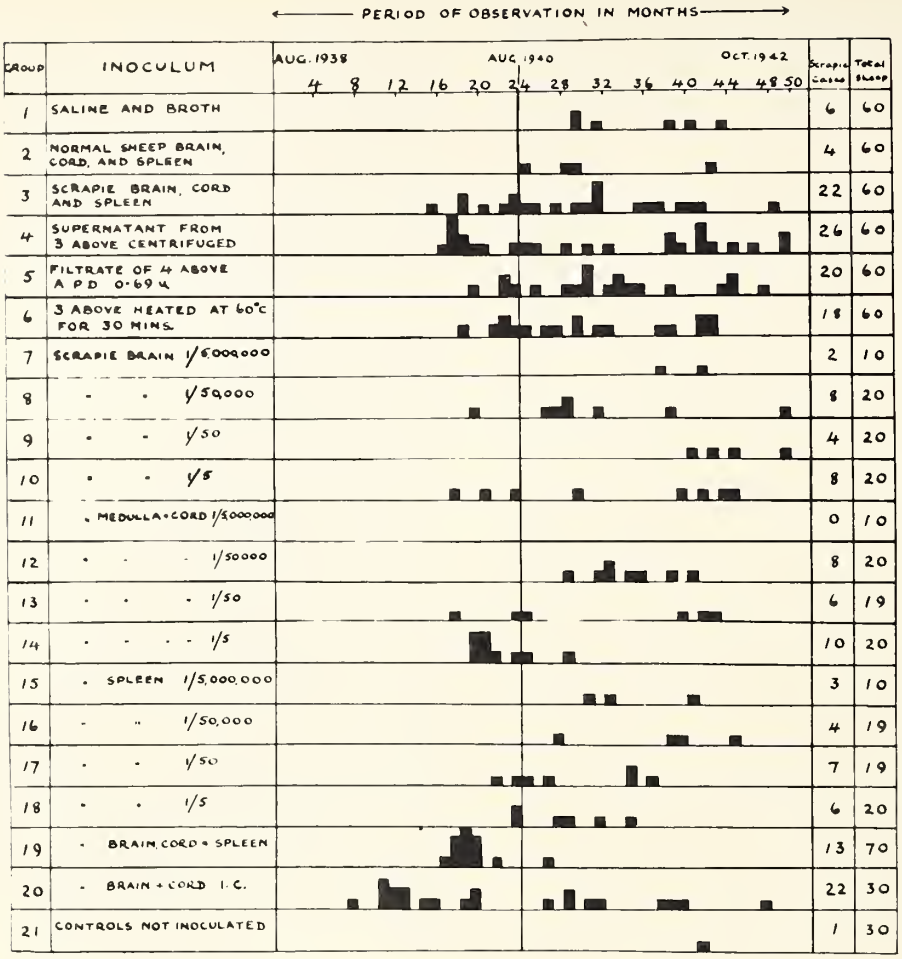

TRANSMISSION OF SCRAPIE
BY LOUPING-ILL VACCINE 1935
BATCH 1
BATCH 2
BATCH 3
NO SCRAPIE
SCRAPIE DEVELOPED IN APPROX. 10 PER CENT OF INOCULATED ANIMALS
NO SCRAPIE
BATCH 2
18,000 DOSES
PREPARED FROM 114 SHEEP 8 WERE YEARLINGS BORN IN 1934 FROM EWES WHICH BEGAN TO DEVELOP SCRAPIE 15 MONTHS LATER IN 1936 TWO SURVIVING LAMBS, FELLOWS TO THE 8 USED FOR VACCINE IN 1935, DEVELOPED SCRAPIE IN 1936/7

Figure 2.

EXPERIMENTAL TRANSMISSION OF SCRAPIE TO SHEEP


PERIOD OF OBSERVATION IN MONTHS
AUG. 1938
AUG. 1940
OCT. 1942
4 8 12 16 20 24 28 32 36 40 44 48 50
GROUP
INOCULUM
Scrapie Cases
Total Sheep
1 SALINE AND BROTH 6 60
2 NORMAL SHEEP BRAIN, CORD, AND SPLEEN 4 60
3 SCRAPIE BRAIN, CORD AND SPLEEN 22 60
4 SUPERNATANT FROM 3 ABOVE CENTRIFUGED 26 60
5 FILTRATE OF 4 ABOVE A P D 0·69 μ 20 60
6 3 ABOVE HEATED AT 60°C FOR 30 MINS. 18 60
7 SCRAPIE BRAIN 1/5000000 2 10
8 " " 1/500000 8 20
9 " " 1/50 4 20
10 " " 1/5 8 20
11 " MEDULLA+CORD 1/5000000 0 10
12 " " " 1/50000 8 20
13 " " " 1/50 6 19
14 " " " " 1/5 10 20
15 " SPLEEN 1/5,000,000 3 10
16 " " 1/50,000 4 19
17 " " 1/50 7 19
18 " " 1/5 6 20
19 " BRAIN, CORD + SPLEEN 13 70
20 " BRAIN + CORD I.C. 22 30
21 CONTROLS NOT INOCULATED 1 30

▪ = 1 sheep affected with scrapie.

Figure 3.

In the first 24 month period after inoculation it will be seen from figure 3 that deaths from scrapie occurred in Groups 3, 4, 5, 6, 8, 10, 13, 14, .17, 18, 19, and 20 in all of which groups it was expected that scrapie infective material would be present in the inoculum. It will be noted that, although heated at 60° C. for 30 minutes, the transmissible agent had not been inactivated and that the filtrate was also capable of setting up the disease. It will also be noted that within 2 years of inoculation no deaths from scrapie occurred in the groups that received saline and broth, normal sheep brain cord and spleen, the high dilutions of infective tissues and the non-inoculated controls.

Contrary to expectation, however, 11 confirmed cases of scrapie developed in the successive 26 months--6 in Group 1, 4 in Group 2, and 1 in Group 21 (the non-inoculated controls). This unexpected result, following a prolonged period of contact between normal

and scrapie-affected sheep at pasture, is analogous with Greig's experience under similar circumstances. Again, many cases of late manifestation occurred as will be seen from the following details of ages of individual animals concerned:

	Age When Exposed to Infection From Animals Inoculated With Infective Material		Age at Death From Scrapie	
	Years	Months	Years	Months
GROUP 1		4	3	8
(Saline		4	2	6
and	2	4	5	7
Broth)	3	4	5	10
	3	4	6	9
	4	4	6	11
GROUP 2		4	2	9
(Normal	1	4	3	11
Brain)	3	4	5	10
	4	4	6	5
GROUP 21 (Control - not inoculated	6	4	9	6

It is difficult to find any explanation for the infection of these animals other than their close association at pasture with experimentally affected animals from other groups.

This experiment provided conclusive evidence that scrapie is a transmissible disease, that the infective agent is filterable, that it can withstand heat at 60°C. for 30 minutes, that the infective agent is present in the brain, spinal cord and spleen of affected animals and that there is a variation in susceptibility to infection, many sheep being peculiarly resistant.

Thus, taking account of the field evidence of earlier observers, especially that of M'Fadyean and Stockman, together with the experimental evidence of Greig and Gordon described in this paper, followed by the experience reported by Stamp and colleagues at Moredun and the spread by contact in mice described by Pattison (8), it may be concluded that, under certain circumstances, scrapie can spread from affected to susceptible animals. In sheep this has been seen only in animals at pasture and its occurrence may sometimes pass unrecognised because of the long period of time that may elapse between exposure to infection and clinical manifestation. While this may not be the most important method of spread of the disease it cannot be disregarded.

REFERENCES

(1) Gordon, W.S., Brownlee, A., and Wilson, D.R.: 1939. 111 Int. Cong. Microb., New York. Report of Proceedings, 1940, 362.
(2) Gordon, W.S.: 1946. Vet. Rec., 58, 516.
(3) Gordon, W.S.: 1957. Vet. Rec., 69, 1324.
(4) Gordon, W.S.: 1959. Proc. 63rd Annual Meeting United States Livestock Sanitary Association, 286.
(5) Stockman, S.: 1913. J. comp. Path., 26, 317.
(6) M'Fadyean, J.: 1918. J. comp. Path., 31, 102.
(7) Greig, J.R.: 1940. Vet. J., 96, 203.
(8) Pattison, I.H.: 1964. Vet. Rec., 76, 333.

DISCUSSION

Dr. Goode:

Do we have any questions or comments regarding Dr. Gordon's paper?

Mr. Yohe:

These cases that might have been caused by field infection showed up very, very late. For instance, the last one I believe you said the ewe was 9 years of age.

If this be true, isn't it pretty difficult to say that any flock is non-scrapie? For instance, you mentioned the Cheviots that you were working with. Are you absolutely certain that there has been no cases of scrapie or could not be in a period working within the 9-year period?

Dr. Gordon:

All I can say about the origin of the sheep is that their source was a sheep farm on which we had been carrying out experiments on the prevention of lamb dysentery; therefore, we were on the farm each year inoculating sheep, and observing all the dead lambs on that farm. The owner was an enlightened man and had he had any cases of scrapie I am sure he would have brought them to our attention.

This was taking place around about 1926 to 1930, and this experiment was set up on that farm in 1938. So I can't, of course, vouch for the fact that this owner did not have any one case of scrapie in his flock between 1930 and 1938, but I feel satisfied that on the inquiries that we were making, we would not have accepted the flock to enter this experiment, had we had the slightest doubt about its freedom from scrapie.

Mr. Yohe:

I wasn't questioning the man's ability to recognize scrapie, but actually he would have had to have had a closed flock for perhaps 10 years before you could say that it had not been introduced by some sheep coming into the flock.

Dr. Gordon:

Yes.

Mr. Yohe:

Was it a closed flock for a long number of years?

Dr. Gordon:

It wouldn't be closed as far as rams were concerned. Rams would be brought in.

Mr. Yohe:

Actually, this could have been brought into these flocks as long as he had been bringing in rams over a period of perhaps as long as 10 years?

Dr. Gordon:

This might be regarded as a possibility, but I think it is highly improbable, because of the history that we had of this farm.

Mr. Parry:

The point of the last question: Were these sheep individually identifiable?

Dr. Gordon:

Throughout the course of the experiment, yes.

Mr. Parry:

No, no, the foundation sheep. I referred to Mr. Yohe's question.

Dr. Gordon:

They were not registered sheep. This is a Cheviot flock. Until we put three marks on the sheep, there was no other identification. We put the mark on at the time of inoculation and followed the history through.

Mr. Parry:

What was the age composition of the flock?

Dr. Gordon:

The age composition ranged from 4 months to 6 years 4 months, and the animals of different ages were randomized as far as possible throughout the inoculated groups in the experiment.

Mr. Parry:

You misunderstand my question. What was the age composition of the flock, between 1930 and 1938, because that is relevant to Mr. Yohe's question.

Dr. Gordon:

The age composition would be the regular ages that occur on a Cheviot sheep hill farm, being in age groups, the youngest 4 months at the time of inoculation, then 1 year 4 months, 2 years 4 months, 3 years 4 months, 4 years 4 months, 5 years 4 months, and 6 years 4 months. That was the age composition of the flock at the time we took it over, and that would be its age composition in the sequence of years between 1930 and '38.

Mr. Parry:

I am sorry you don't understand what we mean by "age composition". That is the qualitative statement of the age. How many animals in the flock were in the age birth group or age group--how many were 3 years and 4 months?

Dr. Gordon:

I can tell you that.

Mr. Parry:

I don't want to press it. It is available and will be coming out in your paper.

Dr. Gordon:

It will.

Mr. Parry:

Is it available for the years 1930 to 1938?

Dr. Gordon:

It wouldn't be. But this is a thing which could be acquired from the owner of the flock. I think it could be obtained because the age composition would be very similar throughout, to the age composition at the time we took it over.

Mr. Parry:

He would have records of 30 years?

Dr. Gordon:

I would say that he would know the number of his different age groups, because this is the kind of record that the hill sheep farmer with that type of stock does keep. He knows the number of 1, 2, 3, 4, 5, and 6-year old ewes he has, and the number that he is planning to cast each year.

Mr. Parry:

He would not know the number of the ewes in each age group?

Dr. Gordon:

The number of the ewes in each age group? Many do, and he might, but without further inquiry it is difficult to say.

Mr. Parry:

If I may leave that, Mr. Chairman, and go to another point. Dr. Gordon, I drew your attention to the age manifestation which we dealt with. I don't want to belabor this now. What I would like to say, to make quite clear, is that this was obtained on two breeder groups which are quite different from Cheviots and Border Leicesters, or Border Leicester Cheviots, which is your half-bred.

I suspect that to reduce criticism, you ought to establish the age of manifestation curve of these breeds. I can't answer this. Before this can be accepted as an important point against the other hypothesis, this will have to be established.

Dr. Gordon:

Perhaps you will be in a better position to establish it for the Cheviot breed than I am. I have yet to determine whether there is much difference between the age of manifestation in different breeds of sheep, so far as I have experienced at the moment, there is little difference in susceptibility to experimental challenge.

Age doesn't seem to matter so far as susceptibility is concerned.

Mr. Parry:

I raise this because I have been shot at by geneticists on this count.

Dr. Gordon:

That is quite understandable.

Dr. Goode:

Are there other questions or comments on Dr. Gordon's paper?

Dr. King:

Dr. Gordon reported to us the experience of two laymen, Mr. "A" and Mr. "B", who had bought sheep from Mr. "X". I wonder, Dr. Gordon, if you have attempted to repeat scientifically, under controlled conditions, the conditions reported to have existed in Mr. "A's" flock and Mr. "B's" flock, to determine whether in fact the experiences are repeatable under scientifically controlled conditions?

Dr. Gordon:

I haven't so far attempted this, because what I have described to you today is experiences that I feel are similar to those that were experienced by those owners and which evidence was produced by Sir John M'Fadyean by close contact and examination of the evidence that these owners could produce.

I feel that Dr. Greig's experiment, which seems to have a lot of criticism thrown at it from time to time, was a complete surprise to me where he had a group of sheep really being exposed in a scrapie environment for a period of 3 years. When you do this for 3 years, you get rather tired of waiting for it

to spread and you begin to believe that it is not going to spread.

It was at this stage that Greig gave these sheep to a sheep farmer. Had he believed that they were going to take scrapie, I doubt very much if he would have taken a risk like that. He gave them to the farmer who accepted them, knowing what their background history was, and he--the farmer--and Dr. Greig, both believed, and I also believed at the time, that these sheep were not going to take scrapie. But 3 months after they had left this scrapie environment, they did develop the disease, and one of the points that I have been trying to establish is that every one of the original animals that developed scrapie fell into the category of late manifestation.

They all developed the disease at a ripe old age, rather indicating that they had become infected after they had reached a considerable age, and roughly between 2 and 5 years of age they picked up the infection and finally came down at a later stage.

So that this convinced me that this disease could spread to susceptible animals in a scrapie-contaminated environment, and I was so satisfied that the disease wasn't going to occur in these sheep and their offspring that, with my eyes open, I took eight of the male lambs, inoculated them with another virus altogether, harvested their brains, made them into a vaccine, and then found that I had in fact transmitted scrapie.

This was a very staggering experience, I can assure you, and rather indicates that the disease can, on occasion--and I don't say it is the most important method--spread from infected to susceptible animals at pasture.

Dr. King:

The two instances, Mr. "A" and Mr. "B", and the Greig experiment, all bear on the point of transmissibility under field conditions?

Dr. Gordon:

Yes.

Dr. King:

Each of those tend to be defective as scientific experiments, in that the first two were observations and recollections of a layman, later reported. The third, the Greig flock, was out from under any scientific surveillance for a period until scrapie developed in the new environment. So that neither of the three can be, per se, considered as proof, but merely as an indication that some work should be done in attempting to repeat. No such work has been done?

Dr. Gordon:

No. the only work that has been done is to bring scrapie-infected animals in contact with susceptible animals indoors.

Until we have a population of sheep which we can guarantee are a 100 percent susceptible, or nearly, it is hardly worthwhile setting up experiments like that, because in any group of sheep you take, you may get a very wide range of difference in susceptibility, a matter which I will point out later in the day.

We have set up various experiments and then found that the proportion of susceptible sheep included was so low that no significant result could be obtained.

So that the question that you would wish to have satisfaction about is one in which you want to expose susceptible sheep in a scrapie environment. This experiment will come in due course, when we have the right population of sheep and the right kind of experimental animals are those likely to be susceptible.

The only evidence I can give you at the moment are those experiences where the disease appeared in sheep that were kept in a scrapie environment, and I think that these kinds of observations will later be confirmed when we have the right kind of sheep with which to do the job.

Dr. Goode:

Dr. Gordon, is it not true that in your presentation immediately following the lunch period, which is a review of the scrapie research at Compton, you will point out that the work that is being taken in step by step fashion, leading to both materials and techniques to accomplish the thing that Dr. King had just alluded to?

Dr. Gordon:

Yes.

Dr. King:

I have one final question. Then you cannot say, as of today, that scrapie is transmissable under field conditions?

Dr. Gordon:

All I can say on the evidence that exists at the moment is that the possibility that scrapie may spread by contact at pasture cannot be excluded.

Dr. Hutt:

I don't want to appear critical. Dr. Greig is a friend of mine and I know he worked under difficult conditions with a difficult disease. Is it not true that he did not have any unexposed controls of similar origin which were transferred at the same time? If he did, I would like to know what was the incidence of scrapie in those controls, how did it compare with the 40 percent which he had.

Dr. Gordon:

The only control would be the flocks from which his sheep were selected. The Cheviot sheep, six of them came from a flock in the southwest of Scotland which happens to be in the ownership of the Chairman of Nature Conservancy in England, and I believe him to be a reliable person. 28 years after Greig purchased these sheep, I inquired very carefully from this owner, and he has not had any cases of scrapie in the 28-year interval.

The half-bred sheep came from a farm in Aberdeenshire, from an area in which scrapie was at that time believed to be absent, and I have since inquired and no scrapie has occurred in the flock of origin of the half-bred sheep.

The interesting thing in Greig's experiment, is that there were two breeds of sheep present, and the disease appeared not in one of these breeds, but in both of the breeds exposed, both in the Cheviots and in the half-breds.

And the further point, is that all of these cases were late manifestations. It seems to me a peculiar coincidence that that should be so.

In my subsequent experiment in transmitting the disease on the farm mentioned, again it is a peculiar coincidence that the sheep of the same age that were inoculated in that experiment were also late manifestations. I am prepared to accept one coincidence, but when it comes to the second coincidence I get a bit more reserved about accepting it and swallowing it hook, line, and sinker. When it comes to a third experience it becomes too difficult for me to accept the fact that scrapie never on any occasion can spread by contact at pasture.

Dr. Hutt:

While this is true, Dr. Gordon, is it not also true that no samples from the unexposed stock were transferred unexposed to the farm on which the scrapie subsequently developed? I point to this only to emphasize the desirability of having complete controls in these subsequent experiments.

Dr. Gordon:

I agree it would have been more perfect if we had had another group of sheep from the same farms held in a scrapie-free environment, and that they hadn't taken scrapie. The only control, of course, is the sheep that were left on the farms of origin.

As I say, 28 years later the best I can do is get a confession from both the owners that supplied these sheep, it was a specific type of inquiry, and they remembered supplying the sheep and also assured me that they had not had cases of scrapie in the interval.

Mr. Yohe:

I believe you mentioned, Dr. Gordon, the average age of sheep coming down with scrapie. Was this for a natural infection? I believe you gave an age which I did not get in my notes. May I have that again, please? Was this for natural infections?

Dr. Gordon:

For natural infections somewhere between $2\frac{1}{2}$ and $3\frac{1}{2}$ years old is the high incidence period, and after $3\frac{1}{2}$ the incidence is dropping. By $4\frac{1}{2}$ as Mr. Parry has already said, at least in his particular population of sheep, he says by $4\frac{1}{2}$ years, 90 percent of cases are going to occur by that age, and 10 percent later.

This I would say from general observation, but not from actual figures that I can quote here at the moment, is typical of other breeds as well.

Mr. Parry:

I am not sure it is quite clear that there are two types of experiments Dr. Gordon has carried out or reported. The one with Dr. Greig is with regard to natural spread, that is, that there is no element of artificial transmission by inoculation.

In the second experiment it deals with artificial post-inoculation. Is that right?

Dr. Gordon:

This is perfectly right.

Mr. Parry:

In view of what we now know about the natural disease in Cheviots and Border Leicesters, I do not think we can separate in the second experiment those cases which are due to natural spread and those cases which are due to artificial post-inoculation spread.

Dr. Gordon:

It is perfectly right, of course, the first experiment, the Greig experiment, is naturally occurring disease. None of these sheep were inoculated. In the second experiment it was an attempt to transmit the disease. So that all of these were inoculated sheep. But the control that developed scrapie was not an inoculated sheep. And those that received saline and broth received material that to me seems to be unlikely to contain the scrapie agent. And the material from normal sheep I felt was unlikely to contain the agent either. So that all of those cases that occurred in these groups were infections from experimentally infected animals, and to all intents and purposes the cases that developed looked entirely like natural cases of scrapie.

Mr. Parry:

The fact that Dr. Gordon has a case in his control group at an age when natural scrapie occurs, of course immediately makes one suspect the genetic background of all these other experimental animals. We can't go any further with this because we don't know their pedigrees.

The other point is how did Dr. Gordon choose his normal animal for inoculation. I have had a lot of trouble over this. That is, does he know the father and mother of that animal, the life history of a considerable number of half-siblings, and how old did the mother and father live?

Dr. Gordon:

I don't know the answer to that. At a later date I will certainly be able to tell you the history of other sheep where we do know.

One point: The animal that developed the disease in the control group was almost 10 years old. It didn't develop the disease at a natural time. It developed it when it was almost 10 years old, which is very ancient, and a very effective-looking late manifestation without inoculation.

Dr. Goode:

We do have with us this morning Mr. Leslie W. Osborne, who is the Agricultural Attache at the British Embassy here in Washington, D.C. I would like for him to stand.

REVIEW OF WORK ON SCRAPIE AT COMPTON, ENGLAND, 1952-1964

W. S. Gordon [1]

In the first paper I presented to this Seminar, I outlined work on scrapie which was carried out at Moredun Institute, Edinburgh from 1932 to 1942. Subsequently, Dr. D. R. Wilson and colleagues, (26) continuing this work, confirmed that scrapie could be transmitted by inoculation from affected to susceptible sheep, that the agent was filterable, that it could be passed indefinitely from group of sheep to group of sheep with cell-free filtrates and that it could withstand boiling and concentrations of formalin that no other virus was known to withstand.

Following this, Dr. Stamp and his colleagues have extended Dr. Wilson's work and Dr. Stamp will be reporting on the progress made.

In 1942, having become Director of the Agricultural Research Council's establishment at Compton, although still very interested in the investigation of scrapie, my active participation was suspended temporarily but, in 1952, when it had been found that some sheep exported from Britain had developed scrapie, it was decided that work on this disease should be initiated at Compton.

Since the Welsh Mountain sheep is small and inexpensive it was decided to utilise this breed. Accordingly, to check that the susceptibility of Welsh Mountain sheep was similar to that of South Scotland Cheviots--a breed already known to provide a good proportion of susceptible animals--8 sheep of each breed were purchased and inoculated with scrapie brain material obtained from Dr. D. R. Wilson of Moredun Institute. None of the Welsh Mountain sheep developed infection, whereas 5 of the Cheviot sheep became affected and were destroyed 5 to 10 months after inoculation.

This was an unexpected result and to check its validity 9 groups of 10 Welsh Mountain sheep were inoculated in May, 1953 with brain material from nine different natural cases of scrapie and a group of 30 was inoculated with material similar to that used in the previous experiment. Out of a total of 120 sheep inoculated, only one animal developed scrapie and that was in the group of 30.

In 1954, the brain from this one affected Welsh Mountain sheep was inoculated into 29 South Scotland Cheviot sheep, 28 Welsh Mountain sheep and 12 goats, producing 14 cases out of 29 in the Cheviot group, 4 cases out of 28 in the Welsh Mountain group, while all the goats proved fully susceptible to scrapie, but with an incubation period considerably longer than that in sheep. (See figure 1, 16)

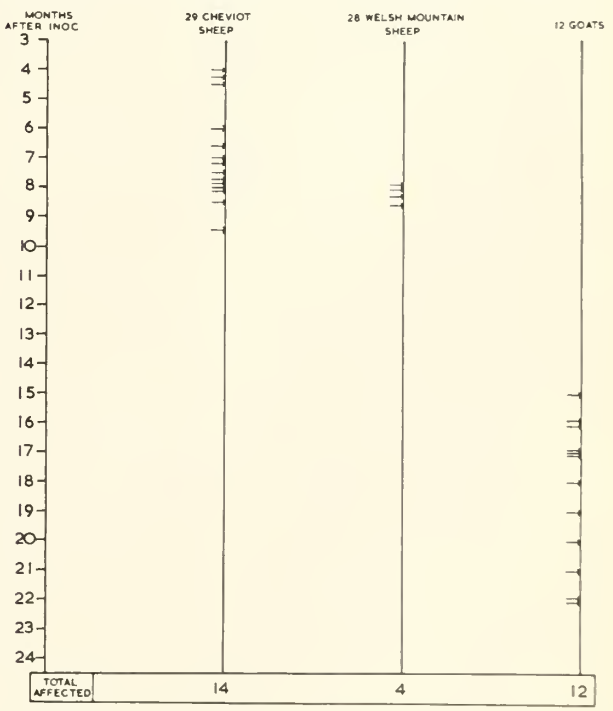


Figure 1.--Development of scrapie in 29 Cheviot sheep, 28 Welsh Mountain sheep, and 12 goats inoculated intracerebrally on 6.8.54 with a 10% brain suspension from a Welsh Mountain sheep.

[1] Director, Agricultural Research Council Field Station, Compton, Berkshire, England.

This experiment further emphasises the unpredictable susceptibility of individuals in any one group of sheep and since the Welsh Mountain breed did not appear to have a high level of susceptibility in the sample tested it was decided to use South Scotland Cheviot sheep in subsequent work.

The fact that the 'take' in goats appeared to be 100 percent was an important step forward because the goat could now be used as an experimental animal with a known susceptibility, enabling much work to be undertaken that was not practicable in sheep.

In September 1955, 198 cast ewes and 4 rams of the South Scotland Cheviot breed were purchased from farms in Scotland known to be free from scrapie. After delivery to Compton, the sheep were divided into four groups and from October 1955 until early January 1956 one of the 4 rams purchased ran with each group. The ewes produced a crop of lambs in the spring of 1956 and on 7th November 1956 the rams were returned to run with the ewes and offspring to produce a crop of lambs in 1957. On 27th November 1956, 20 days after the rams had been put to the ewes, the 4 rams plus 83 ewes and their 92 lambs, which could be identified in their family groups beyond doubt, were segregated. They were inoculated subcutaneously with 5.0 ml. of 10 percent suspension of 1956 scrapie sheep brain pool from Moredun Institute and, after 27th November, were run together as an infected flock.

The remaining animals, which comprised 77 ewes and 69 lambs, acted as normal controls and were grazed on a separate pasture, to which inoculated or scrapie-affected sheep had never had access.

The purpose of this experiment was to relate the susceptibility of parents and their offspring and this aspect of the result will be dealt with in my subsequent paper. For the moment, it is sufficient to mention that scrapie occurred in a proportion of the inoculated parents and their offspring, while the controls remained free from the disease.

The point I want to make concerns the second crop of lambs. It will be recalled that for 20 days prior to inoculation and the formation of separate infected and normal flocks, the rams had run with the ewes and their 1956 lambs. None of the resultant lamb crop, born in the spring of 1957, was inoculated yet 9 lambs

PARENTS

INOCULUM	EWES	RAMS
10% SCRAPIE BRAIN SUSPENSION IN SALINE	140	4
AFFECTED WITH SCRAPIE	45	2

OFFSPRING

TOTAL BORN	59	64
AFFECTED WITH SCRAPIE	9	2

Figure 2.--Occurrence of scrapie in the progeny of inoculated parents.

developed scrapie when they were between 7 and 18 months of age and another became affected when it was about 2 1/2 years old. Thus, 10 of the non-inoculated progeny from inoculated parents took scrapie. (6)

This was an unforeseen development--a byproduct of the original experiment--which had been designed to obtain more information on the pattern of susceptibility where father, mother, and offspring are all exposed to scrapie infection by inoculation. Once the animals, having been positively identified in their family groups, had been inoculated, there were many practical advantages in running them together as an infected flock, rather than making special arrangements for their maintenance in four separate groups. With the unexpected development of scrapie in some of the 1957 lamb crop, however, it was very much regretted that the four groups had not been kept separate. It would be particularly interesting to know if either or both of the rams which eventually took scrapie had sired the non-inoculated lambs which developed the disease but, as things are, any one of the four rams could have been responsible.

To confirm that this was not a chance result, it was decided to run another group experiment using 68 ewes from the normal flock, to be divided into six groups, each group with a ram, thus ensuring that on this occasion both parents of the resultant progency could be identified.

Five South Scotland Cheviot rams were purchased from clean farms in Scotland and the sixth was home-bred, the son of one of the

noninoculated original ewes, sired by one of the rams which had proved susceptible to scrapie in the previous experiment. All the ewes and rams were inoculated on 18th November 1958 and the ewes were then divided into six groups, one ram running with each group. The resultant lamb crop, born in the spring of 1959, was not inoculated and, of the 63 lambs born, 3 developed scrapie when 7, 12, and 18 months of age, respectively.

As a result of these experiments it may be concluded that the progeny of inoculated parents, although themselves not inoculated, can develop scrapie, though the method of transmission from parents to offspring is still obscure.

Since it had become obvious that there was a wide range of variation in susceptibility in different groups of sheep it was decided to determine the comparative susceptibility to experimental scrapie of sample flocks of 24 different British breeds of sheep. Each sample flock comprised approximately 30 ewes and a ram, which were placed in pens in isolation and allowed to breed. Crops of lambs were produced in 1956 and 1957. All the ewes and a proportion of their male and female lambs were removed from isolation in July 1957 and tested for susceptibility by inoculation with the 1956 scrapie sheep brain pool from Moredun Institute. The result of this test is shown in figure 3 (6, p. 286). It will be seen that there was a wide range of variation in susceptibility between breeds ranging from 78 per cent in the Herdwick sample to nil in the sample of Dorset Downs. Not only was there a variation between the different breeds, but there was also a difference in susceptibility in families within breeds. For example, the Leicesters were recruited from six different

COMPARATIVE SUSCEPTIBILITY TO SCRAPIE IN SAMPLES OF 24 BREEDS OF SHEEP

INOCULUM 10% SUSPENSION OF SCRAPIE BRAIN TISSUE IN SALINE, 5.0 ml. SUBCUTANEOUSLY

BREED	NUMBER TESTED	AFFECTED with SCRAPIE	PER CENT
HERDWICK	36	28	78
DALESBRED	43	31	72
SWALEDALE	46	25	54
S.S CHEVIOT	45	16	36
DERBY. GRITSTONE	46	16	35
EXMOOR HORN	41	14	34
BORDER LEICESTER	42	11	26
SCOT. BLACKFACE	44	8	18
SOUTH DEVON	35	6	17
ROMNEY MARSH	43	7	16
WELSH CHEVIOT	40	6	15
RYELAND	34	5	15

BREED	NUMBER TESTED	AFFECTED with SCRAPIE	PER CENT
DORSET HORN	45	6	13
SUFFOLK	51	6	12
LEICESTER	42	5	12
WELSH MOUNTAIN	42	4	10
HAMPSHIRE DOWN	30	3	10
N.S.CHEVIOT	45	4	9
SOUTHDOWN	38	3	8
WILTSHIRE HORN	57	4	7
SHROPSHIRE	41	2	5
KERRY HILL	41	1	2
CLUN FOREST	52	1	2
DORSET DOWN	48	0	0

INCUBATION PERIODS FROM TIME OF INOCULATION RANGED FROM 3½ TO 23 MONTHS

PERIOD OF TEST : JULY 1957 TO JULY 1959

Figure 3.

flocks and susceptibles were found in animals from three of the source flocks whereas those from the three other source flocks were completely resistant. This type of result was not confined to one breed but was apparent in all the breeds where animals had been obtained from a number of different flocks.

As a result of this experiment, it was concluded that there would be value in breeding sheep at the two ends of the susceptibility spectrum and, by a process of selection based on classification for susceptibility, gradually to build up a highly susceptible population at the Herdwick end of the scale and a highly resistant one at the Dorset Down end. This long-term breeding programme is now in progress at Compton and if success attends the effort it will provide a population of experimental animals that will facilitate work aimed at clarifying the epidemiology of scrapie infection.

The results of transmission of scrapie by ingestion to goats and sheep have been described by Pattison and Millson (19, 3).

At the time that paper was published, of 50 sheep dosed orally with scrapie sheep brain 7 had developed scrapie, whereas none of the 46 controls, dosed orally with normal brain, developed the disease.

Subsequent to publication, a further sheep from the group dosed with scrapie brain developed scrapie. This was a Derbyshire Gritstone and the incubation period in this case was 13 months.

Six months after this last scrapie case developed, i.e. 19 months after the initial dosing, no further cases had occurred and the experiment was discontinued. It was decided, however, to test the susceptibility to scrapie of the survivors and of the control animals prior to disposing of them. Accordingly, on 16th June 1961, 37 of the sheep which had previously received scrapie brain and 44 of those which had received normal brain were inoculated subcutaneously with 5 ml. scrapie sheep brain suspension.

As will be seen from table 1 no cases of scrapie appeared in the animals which had survived the oral dosing with scrapie sheep brain, suggesting that all susceptible animals had been detected by the oral dose.

In the control group, tested with scrapie material for the first time, 10 cases of scrapie developed.

Table 1.--Infection of sheep with scrapie by the oral route

Breed	Number dosed orally with scrapie brain	Cases of scrapie	Inoc. with scrapie brain 19 mths. later	Cases of scrapie	Number dosed orally with normal brain	Cases of scrapie	Inoc. with scrapie brain 19 mths. later	Cases of scrapie	Incubation period (Months)	
Clun Forest.....	1	--	1	--	1	--	1	--		
Dalesbred.....	1	1	--	--	--	--	--	--	$7\frac{1}{2}$	
Derbyshire Gritstone.....	3	2	1	--	3	--	3	3	$5\frac{1}{2}$:13	10:11:12
Dorset Down.....	2	--	2	--	1	--	1	--		
Dorset Horn.....	4	--	3	--	3	--	3	--		
Exmoor Horn.....	1	--	1	--	2	--	2	--		
Hampshire Down.....	2	--	2	--	3	--	3	--		
Leicester.....	1	--	1	--	1	--	1	--		
North Scotland Cheviot.....	8	--	8	--	7	--	7	1		11
Romney Marsh.....	3	--	3	--	3	--	3	--		
Ryeland.....	2	--	2	--	2	--	2	--		
Scottish Blackface.....	1	--	1	--	1	--	1	--		
Shropshire.....	1	--	1	--	1	--	1	--		
Southdown.....	2	--	2	--	1	--	1	--		
South Devon.....	2	--	2	--	2	--	2	--		
South Scotland Cheviot.....	3	3	--	--	2	--	2	2	9:10:10	8:11
Swaledale.....	4	1	1	--	4	--	3	2	$10\frac{1}{2}$	6:10
Suffolk.....	3	--	2	--	3	--	3	--		
Welsh Mountain.....	2	--	2	--	2	--	2	1		18
Wiltshire Horn.....	4	1	2	--	4	--	3	1	$7\frac{1}{2}$	11
Totals	50	8	37 ø	--	46	--	44 +	10	Range $5\frac{1}{2}$-13	6-18

øIn addition to 8 deaths from scrapie, 5 resulted from other causes. +2 deaths from causes other than scrapie.

This experiment illustrates the ease with which the scrapie agent can infect a susceptible animal and the oral route may yet prove to be an important portal of entry under natural conditions.

In 1959 it was decided to determine once again that intracerebral inoculation of normal sheep brain did not produce scrapie and a number of breeds was available for this purpose.

The sheep used were 127 ewe and wether lambs, born and reared in isolation, the non-inoculated progeny of rams and ewes used in the 24 breed experiment.

All 127 animals were transferred to buildings in the isolation compound which are set aside for housing scrapie-affected animals and had been used for this purpose for some considerable time.

Only those breeds could be used in which 8 lambs were available as 4 lambs were to be injected intracerebrally with 1.0 ml. of a 10 percent suspension of normal sheep brain and 4 controls were to receive 1.0 ml. of normal physiological saline. In the event, 16 breeds were available but, as there were only 7 Border Leicesters, their control group consisted of 3 animals instead of 4.

The normal sheep brain for the inoculum was derived from two of the most scrapie-susceptible and two of the most scrapie-resistant breeds, as evidenced by the susceptibility shown in the results of the 24 breed experiment.

One lamb of each of the 16 breeds used was inoculated with Swaledale normal brain, one with Dalesbred normal brain, one with Dorset Down normal brain, and one with Clun Forest normal brain while the 4 controls of each breed were inoculated with saline.

As will be seen from table 2 none of the animals of any breed or group developed scrapie between the inoculation date (28th October 1959) and the discontinuation date (16th June 1961)--a period of 20 months. Before disposing of these sheep, however,

Table 2.--Inoculation of normal sheep brain

Recipient breeds	Donor inoculum				Controls (Saline)	Inoculated scrapie brain 20 months later	Scrapie cases	Range of Incubation period (Months)
	Swaledale Normal Brain	Dalesbred Normal Brain	Dorset Down Normal Brain	Clun Forest Normal Brain				
Border Leicester...	1	1	1	1	3	7	-	5-11
Clun Forest.....	1	1	1	1	4*	7	--	--
Dalesbred.....	1	1	1	1	4	8	-	7-13
Derbyshire Gritstone.....	1	1	1	1	4*	7	3	6-11
Dorset Down.....	1	1	1	1	4	8	--	--
Dorset Horn.....	1*	1*	1*	1	4	5	--	--
Hampshire Down.....	1	1	1*	1	4	7	--	--
Kerry Hill.....	1	1*	1	1	4	7	--	--
Leicester.....	1	1	1	1	4	8	--	--
North Country Cheviot.....	1	1	1	1	4	8	1	9
Romney Marsh.....	1	1	1	1	4*	7	--	--
South Country Cheviot.....	1	1	1	1	4*	7	-	7-11
Suffolk.....	1	1	1	1	4*	7	--	--
Swaledale.....	1	1	1*	1	4	7	4	6-11
Welsh Mountain.....	1	1	1	1	4	8	1	12
Wiltshire Horn.....	1	1	1	1	4	8	3	9
Totals	16	16	16	16	63	116	[illegible]	[illegible]

NO CASES OF SCRAPIE UP TO HERE

*Died from causes other than scrapie: *in Controls indicates death of one animal only.

it was decided to test their susceptibility to scrapie by subcutaneous inoculation with 5 ml. scrapie sheep brain suspension. The sheep were inoculated on 16th June 1961 and 27 cases of scrapie developed before the animals were finally disposed of for slaughter in January 1963--19 months later.

As a result of this experiment it may be concluded that it would be unusual if normal sheep brain was ever capable of producing scrapie unless of course, the animals of origin were in the incubative stage of a scrapie infection.

Apart from the work, which has been in progress using sheep, the work on scrapie at Compton is concentrated in three departments. The first, which had been involved for the longest period, is the Pathology Department, headed by Mr. I. H. Pattison, who has a long list of publications on the subject, especially involving the use of goats.

Second is the Virology Department, under Dr. D. A. Haig, whose main endeavour is to cultivate the scrapie agent and to characterise it more precisely.

Thirdly, the Biochemistry Department, under Dr. G. D. Hunter, is also concerned with the more precise characterisation of the scrapie agent and is making the biochemical approach to its purification, while work on the transmission of scrapie to small laboratory animals has been pioneered by Dr. R. L. Chandler.

It is only possible to select some of the highlights of the contribution of these workers to the solution of the scrapie problem and perhaps this can be illustrated best in a series of tables depicting extracts from some of their work.

Fig. 4 shows this is the second passage in goats and from goats back to sheep. From the first goat to which the disease had been transmitted brain material was passed into 10 Cheviot sheep and 16 goats and from the second affected goat, 10 Cheviot sheep and 10 goats were inoculated. (16)

It will be seen that while only a proportion of the sheep became affected, all the goats inoculated developed scrapie with incubation periods ranging from about 7 months to 21 months.

In the course of passing the scrapie agent in series through goats, Pattison was able to

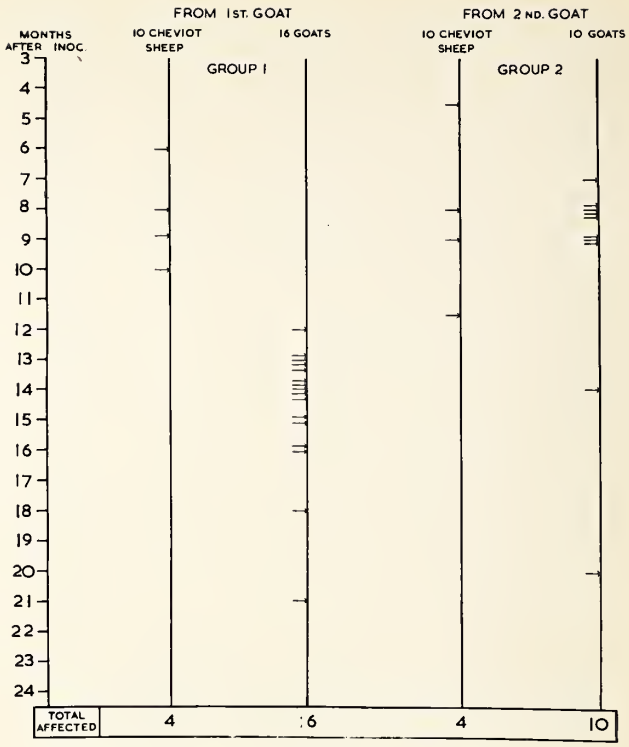


Figure 4.--Sub-inoculations from the first two goats affected.

distinguish two distinct syndromes--one, which has been variously called the nervous, sleepy or drowsy syndrome and the other, the scratching syndrome. (figures 5 and 6). With the brain material obtained he has been able to pass these two types of syndrome in series through goats.

Figure 5.--Goat affected with the 'drowsy' syndrome. Note the abnormal posture, with the animal leaning to the left. There is no evidence of scratching. (18)

Figure 6.--Goat affected with the 'scratching' syndrome. Note scratched areas over neck, shoulders, back and rump. (18)

Figure 7 demonstrates the relative usefulness of sheep and goats for diagnosing scrapie in brain material obtained from natural cases of the disease. (17)

Five natural cases of scrapie in Swaledale sheep have been passed in each case into sheep and goats and it will be seen that while only a proportion of sheep developed infection, all goats inoculated, with the exception of one from Sheep 4, have developed scrapie. This exemplifies the economy in animals if goats are used for diagnostic purposes but it has the disadvantage that the incubation period in goats, especially on the first passage of material from sheep to goats, can be very prolonged.

Figure 8 has been prepared to show that both sheep and goats maintained in direct contact with a succession of sheep and goats affected with experimentally produced scrapie have failed to contract the disease after exposure for periods up to 5 years. Since the

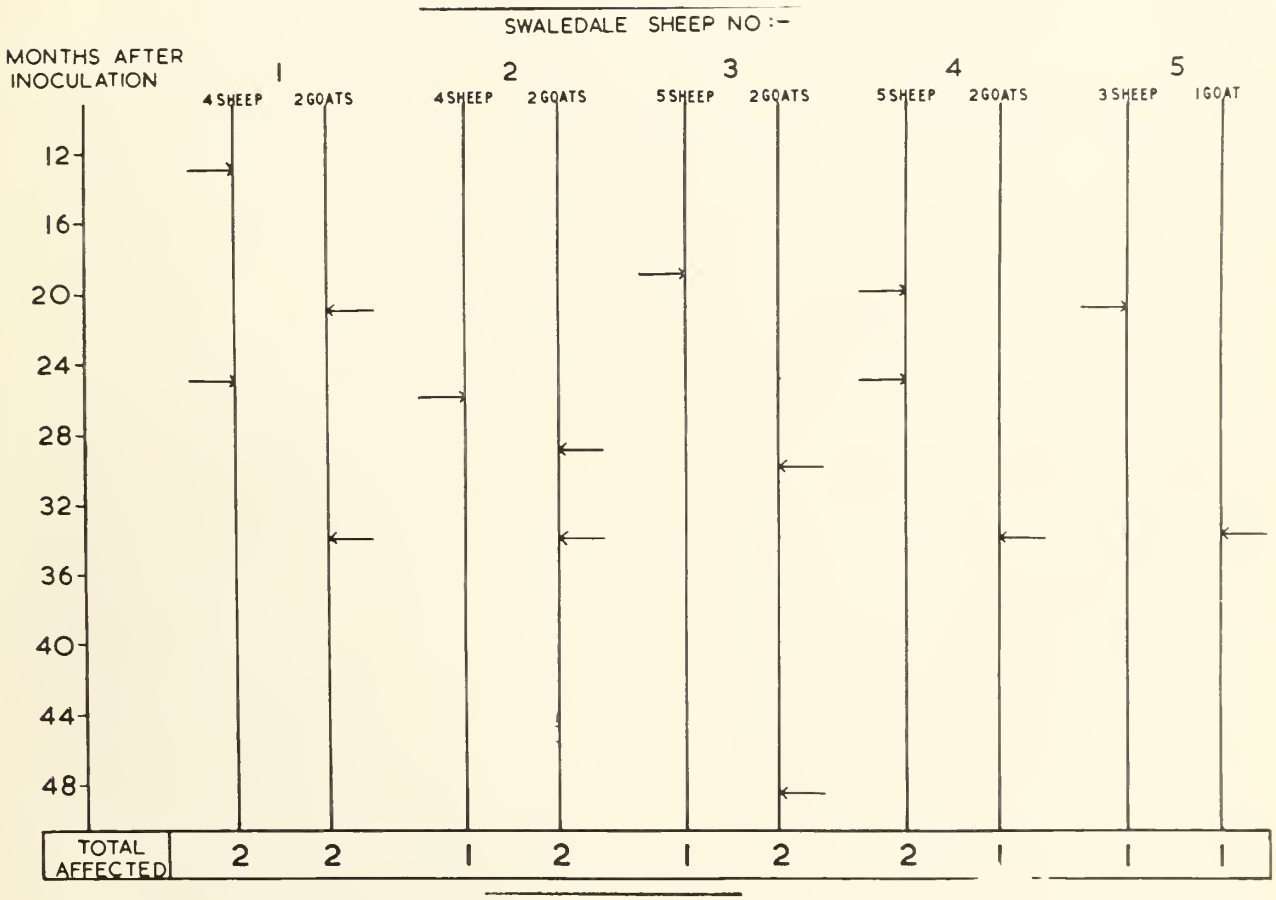


Figure 7.--Intracerebral inoculation of sheep and goats on 27.11.56 with brain suspension from five scrapie-affected Swaledale sheep.

Description	Total number of animals	Number of animals in contact with scrapie for various periods (years)			
		1 - 2	2 - 3	3 - 4	4 - 5
Not inoculated	37 goats 18 sheep	3 0	17 18	7 0	10 0
Inoculated with materials that did not produce scrapie e.g. saline, milk, urine, etc.	155 goats	43	96	14	2
Total	210	46	131	21	12

Figure 8.--The failure of scrapie to spread among housed goats and sheep. (21)

figure was prepared the sheep have been in contact for 55 months without becoming affected.

Figure 9 shows the result of inoculating goats with brain material, saliva, milk, urine and faeces both from scrapie-affected and normal goats and also the effect of feeding the scrapie agent to goats. (19)

It will be seen that while the brain material from the affected goat was capable of transmitting the disease, none of the secretions or excretions was capable of doing so and all the material from the normal goat proved negative. Considerable interest was aroused by the successful production of scrapie in 2 out of 2 goats that received the scrapie agent by the oral route.

Figure 10 shows the result of inoculating various tissues from donor goats (21).

All donor goats were inoculated intracerebrally at the same time with scrapie goat brain suspension and, at intervals after inoculation (as shown in figure 10 on page 27), the donor animals were killed and each of the tissues listed was inoculated into one recipient goat. It will be seen that one day after inoculation the transmissible agent was detectable in the brain, cerebrospinal fluid and pituitary gland, with incubation periods up to 621 days. There was a general tendency for the incubation period in recipient animals to be longer when material from donors in the early preclinical stages of the disease was used. It was thought that this might reflect a lesser concentration of the scrapie agent in the tissues of the early donors and a higher concentration of the agent in those that had reached clinical manifestation.

It is generally accepted that the agent is very resistant to heat and figure 11 illustrates the result obtained after boiling the transmissible agent (20). It will be seen that 5/6 goats became affected after the material had been boiled for 3 hours. It will be noted also that one of the goats inoculated with autoclaved material is shown in the figure with a question mark, which indicates that it exhibited clinical signs of scrapie for several months, after which it made a complete recovery.

The peculiar resistance of the agent to formalin is also well known and figure 12 illustrates that the agent can resist 8 per cent formalin without being inactivated.

It has since been shown that it can withstand 10 percent formalin for 28 months and still remain infective.

EXPERIMENT 47.

INTRACEREBRAL INOCULATION OF GOATS ON 14.5.58. WITH VARIOUS SECRETIONS AND EXCRETIONS FROM A SCRAPIE-AFFECTED GOAT OR FROM A NORMAL GOAT

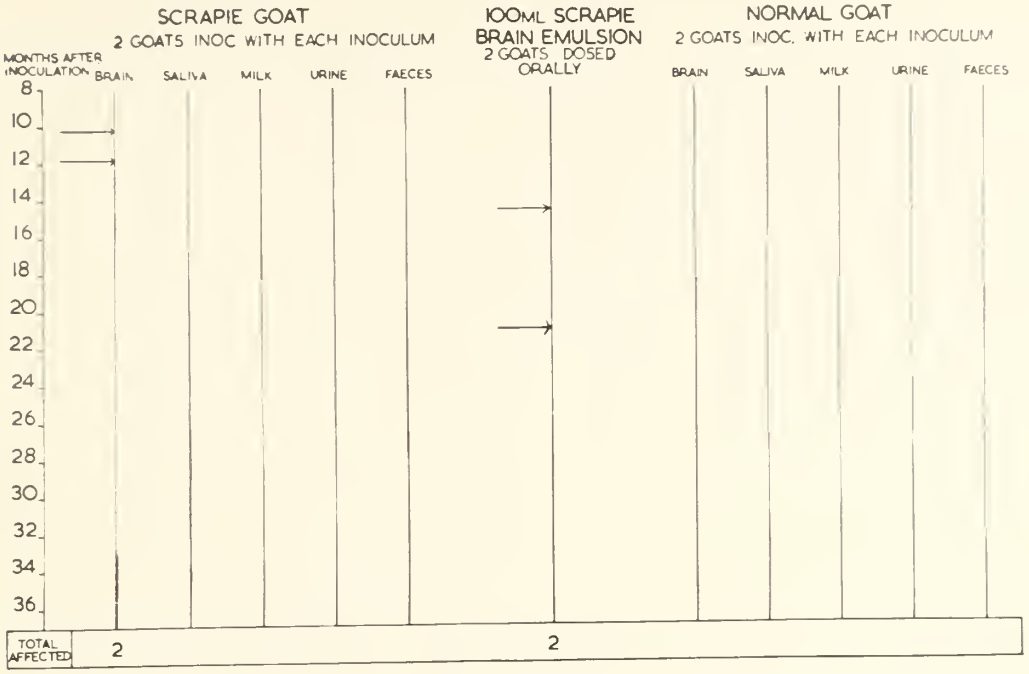


Figure 9.

SUBINOCULATIONS OF VARIOUS TISSUES FROM DONOR GOATS INOCULATED I.C. ON 3.6.58 WITH SCRAPIE GOAT BRAIN SUSPENSION

DONOR NUMBER	KILLED/ DAYS AFTER INOCULATION	SYMPTOMS OF SCRAPIE	APPEARANCE OF SCRAPIE (DAYS AFTER INOCULATION) IN RECIPIENT GOAT INOCULATED WITH -									
			BLOOD	CSF	URINE	BRAIN	SCIATIC NERVE	PITUITARY	ADRENAL	MUSCLE	SALIVARY GLAND	NON-INOC IN CONTACT
1	1	NO		495		621		461				
2	8	NO				365						
3	15	NO		376		344						
4	22	NO				323		614				
5	36	NO				323		336				
6	56	NO				342			DNS 768			
7	92	NO				DNS 26		316				
8	121	NO		341		260	354	241				
9	147	NO				232		323			508	
10	176	NO		306		222		264	523		502	
11	217	NO				334	516	266	334		494	DNS 390
12	243	YES	DNS 457	281		224	385	322	447		385	
13	279	YES		DNS 211		236	398	251	293			
14	344	YES		573		271	305	369	305	445	334	

DNS=DIED NOT SCRAPIE

Figure 10.

INTRACEREBRAL INOCULATION OF GOATS ON 24.9.59 WITH HEATED OR UNHEATED SCRAPIE GOAT BRAIN SUSPENSION.

6 GOATS INOC. WITH EACH INOCULUM.

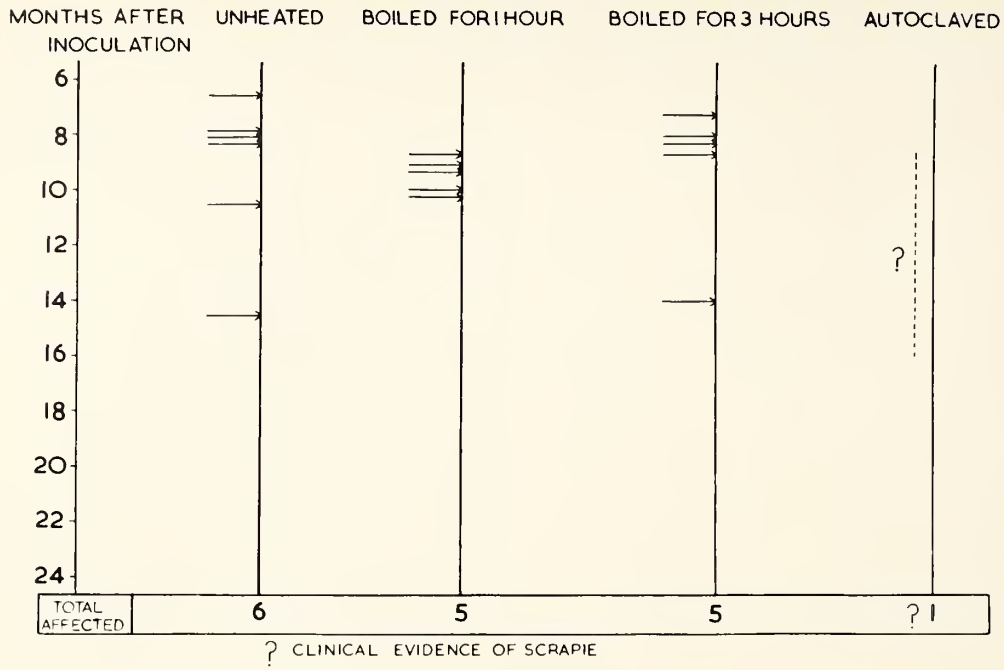


Figure 11.

EFFECT OF FORMALIN ON SCRAPIE GOAT BRAIN

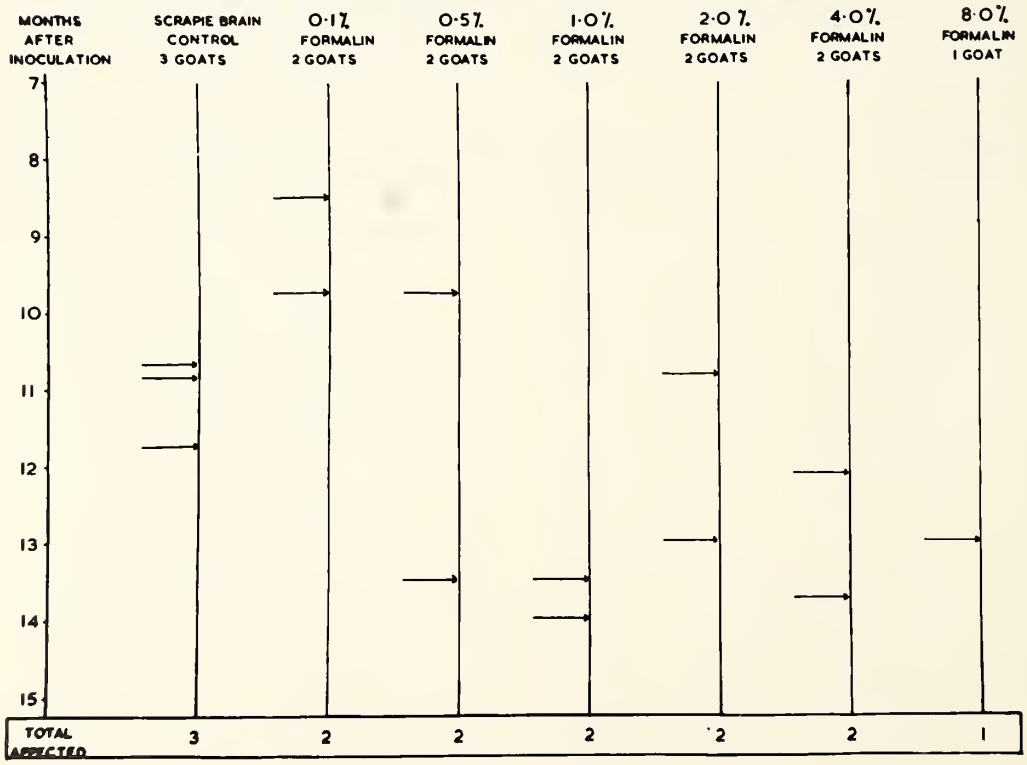


Figure 12.

Figure 13 shows that the agent can also withstand freezing and thawing up to 34 times without being inactivated (18).

Figure 14 shows the infectivity of various dilutions of scrapie goat brain from which it will be seen that the material was infective at the highest dilution, the end point not having been reached (20). It seems probable that the end point may be approximately a dilution of 10^{-5}.

An important advance in the study of the nature of the infective agent was made by Chandler at Compton and reported in 1961, when he succeeded in transmitting the disease to mice. A possible reason for Chandler's success, where previous workers had failed in infecting small animals, was the fact that the 'drowsy' goat strain, which resulted from a long series of passes of Wilson's strain intracerebrally through sheep and then by Pattison through goats, had become adapted to take more readily in mice. It is much more difficult to adapt either the sheep strain or the 'scratching' goat strain to mice but success with the 'drowsy' goat strain encouraged more persistent attempts resulting in the agent being successfully passed direct to mice from affected sheep and 'scratching' goats. Lesions in the nervous system of mice consist of punched out vacuoles in the brain stem adjacent to the cerebellum, as shown in figure 15, also vacuoles in the hippocampus (figure 16), with an accompanying astrocytosis of the thalamus as shown in figure 17, and astrocytosis in the spinal cord (see figure 18).

Further details of the histopathology of the disease in mice have been described by Pattison and Smith (24).

More recently, Chandler has succeeded in infecting rats.

The importance of having established the disease in small laboratory animals lies in

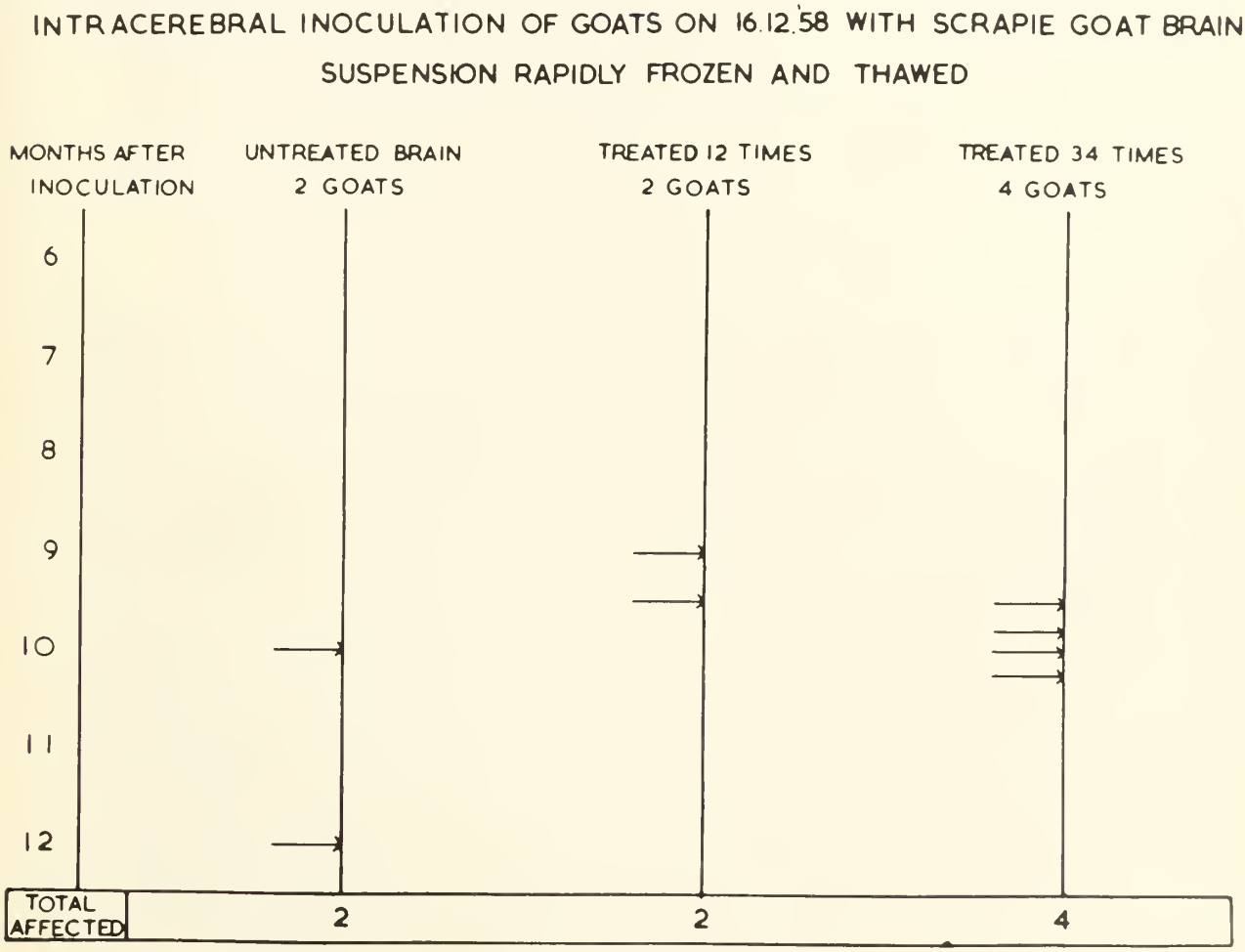


Figure 13.

INTRACEREBRAL INOCULATION OF GOATS ON 13.6.59 WITH VARIOUS DILUTIONS OF SCRAPIE GOAT BRAIN

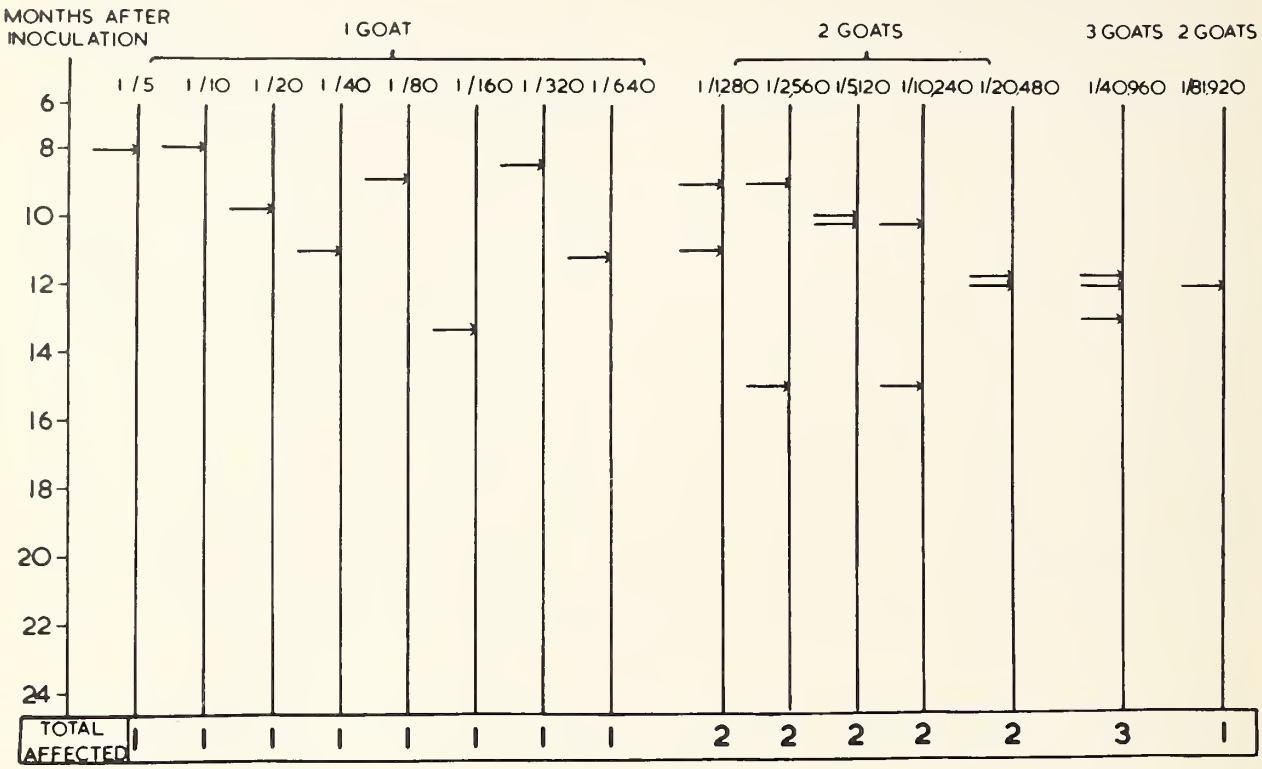


Figure 14.

the fact that it has made the scrapie transmissible agent available to research workers in many other laboratories and it is hoped that their endeavours will be helpful in characterising the transmissible agent more precisely.

Another recent and important observation has been made by Pattison (see figure 19) (15).

This figure depicts the occurrence of scrapie in non-inoculated mice kept in contact with scrapie-affected mice.

Two groups each of 60 mice were housed together--60 being inoculated and 60 non-inoculated. After 5 1/2 to 6 1/2 months the inoculated mice developed scrapie and were replaced by a second group of inoculated mice which, in turn, developed scrapie between 11 and 12 months after the start of the experiment. When the second group of inoculated mice was introduced it was resented by the noninoculated group and a considerable amount of fighting took place, resulting in a number of deaths. Eight and one-half months after the commencement of the experiment one of the in-contact mice developed scrapie and eventually, a total of 15 became affected (figure 19).

This provides further evidence in support of the view that under certain circumstances scrapie can develop in healthy animals maintained in a scrapie contaminated environment. Because fighting occurred among animals in this experiment and because it has already been shown that scrapie can be transmitted orally, it could be argued that the disease may have spread to the noninoculated mice by ingestion of tissue fragments containing the transmissible agent. Nevertheless, it will be noted from the incubation period of the first in-contact mouse to develop infection that it probably contracted the disease from the first group of inoculated mice with which it was associated.

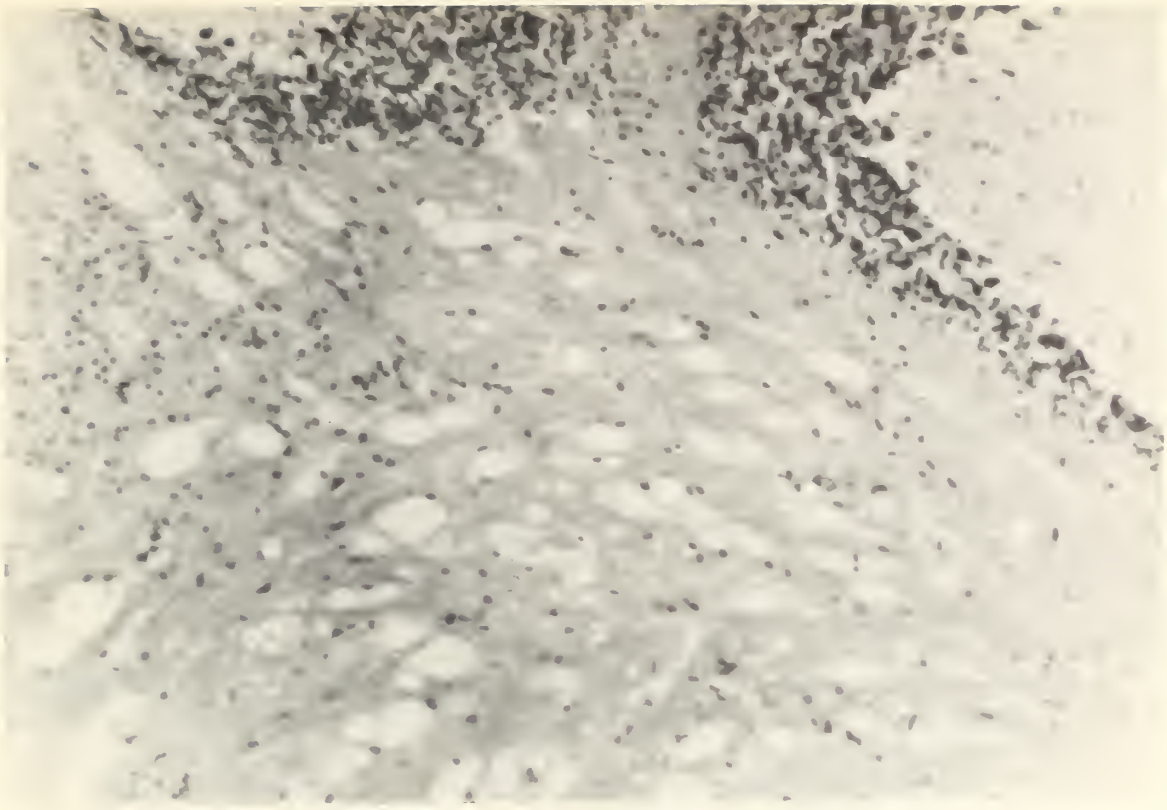


Figure 15.--Photomicrograph of vacuoles, mouse brain.

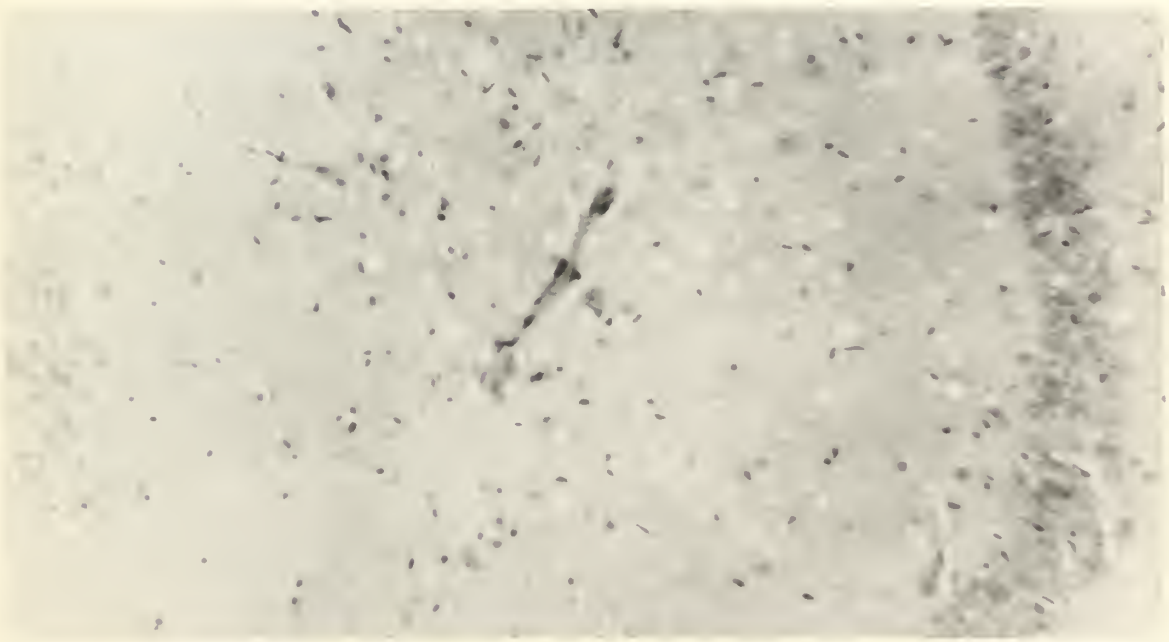


Figure 16.--Photomicrograph of vacuoles in the hippocampus, mouse brain.

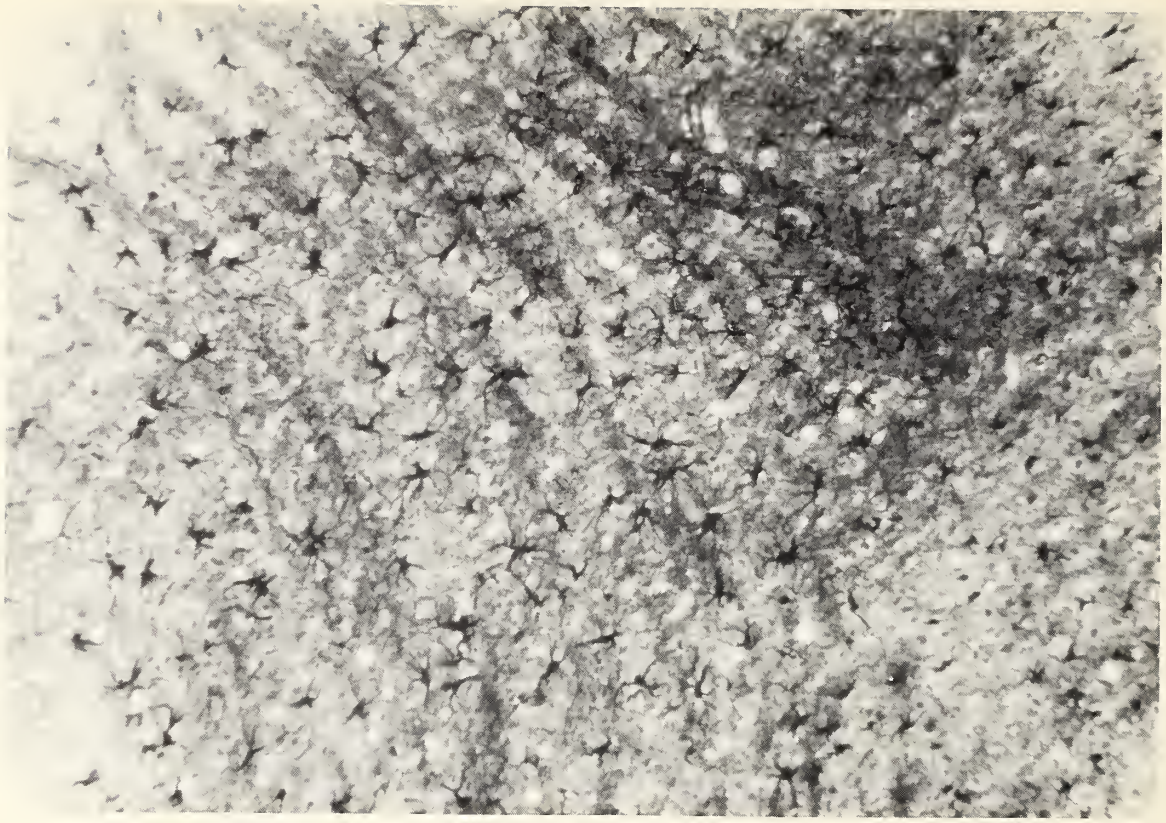


Figure 17.--Photomicrograph of astrocytosis of the thalamus, mouse brain.

Figure 18.--Astrocytosis in spinal cord.

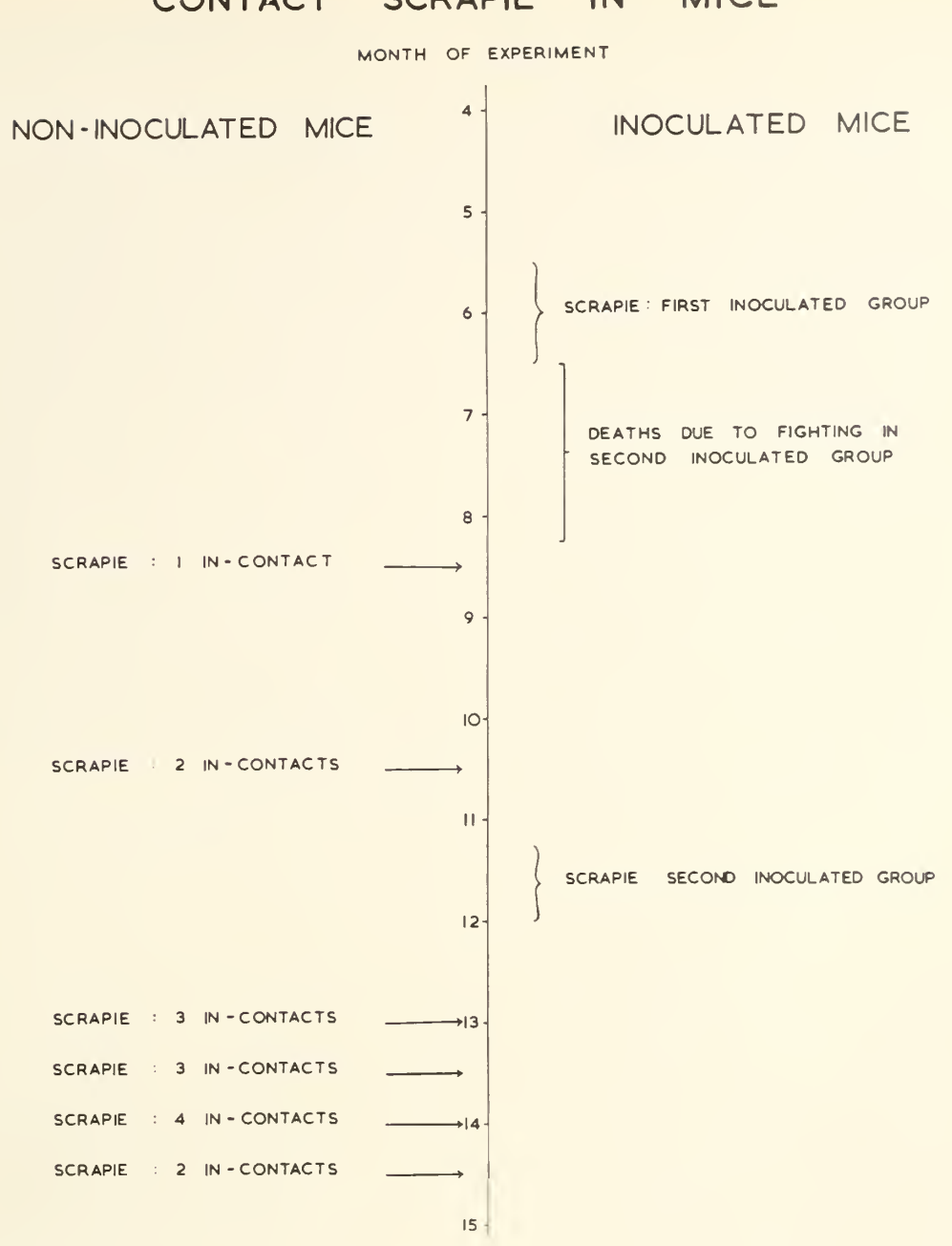


Figure 19.

I am indebted to Dr. D. A. Haig and Mr. M. C. Clarke for the following notes on their endeavours to propagate the scrapie agent in tissue culture.

In spite of the apparent wide host range and tissue affinity of the scrapie agent there are no published reports of its cultivation in tissue cultures. At Compton a large number of attempts at such propagation have been made. This work is still in progress and, while some interesting results have been obtained, their significance is uncertain.

Although a wide variety of cell cultures have been used, only those prepared from kid-kidneys or mouse-embryos have given any indication of promise. Kidneys were taken

from goat-kids born at Compton when 1 to 7 days of age. Mouse embryos were taken during the last stages of pregnancy. The tissues were trypsinised, washed and suspended in Earle's balanced salt solution containing 0.5% lactalbumin hydrolysate, 0.1% yeast extract and 10% calf serum. Once monolayers had formed the serum content was reduced to 5%. Incubation was at 37° C and passages to freshly prepared cells were made usually at 14 to 28 day intervals.

For passage and animal inoculation, cultures were frozen and thawed and the suspension was either transferred to fresh cultures or inoculated intracerebrally into animals. Goats received 1.0 ml. while mice received 0.05 ml. Brains were collected only from mice or goats that had been killed. They were preserved either at -60° C or in 10% formalin.

When determining dilutions of original brain material present in the tissue cultures, only dilutions made at time of passage were considered; no account was taken of the numerous fluid changes that were made.

In a series in which scrapie mouse brain suspension was passaged in mouse-embryo cells at 14 day intervals, it was noticed that from the fourth passage onwards, cytopathic effects were apparent in the cultures. In stained preparations of these cells bodies resembling mycoplasms could be seen. Passage was then continued at 5-day intervals. Material taken at various passage levels was inoculated into mice and goats. Briefly, the reactions observed were as follows:

(a) 5th Passage In mice inoculated with material from the 5th passage in tissue cultures (dilution of original inoculum greater than 10^{-6}) 9 mice of 19 examined from 4 to 12 months after injection showed lesions of encephalopathy. These were characterised by vacuolation of the ground substance of the brain to an extent greater than seen in normal mice, but only in two cases sufficiently marked to be indistinguishable from published descriptions of scrapie in the mouse.

(b) 6th Passage Material from the sixth serial passage (dilution of original inoculum not less than 10^{-7}) was inoculated into 2 goats. One died of an undiagnosed condition soon after inoculation, but the other showed C.N.S. abnormality 9 months later. Histological examination of the brain revealed extensive, bilaterally symmetrical vacuolation of the ground substance.

(c) 7th Passage Serial ten-fold dilutions were prepared from material from the seventh passage (dilution of original inoculum not less than 10^{-8}) and were inoculated into mice from 10^{-3} through 10^{-7}. Only the mice that received the 10^{-3} dilution have been examined at present. Of these, one was apparently normal, 4 showed lesions only slightly more marked than any seen in normal mice, while 3 showed changes almost as marked as those shown in typical mouse scrapie.

Similar changes have been observed in brains from mice inoculated with 5th passage levels of two other series of passages in one of which sheep brain and the other sheep spleen were passed in mouse embryo cells and in still another series in which mouse brain was passaged in kid-kidney cells. In none of these passages was a P.P.L.O. observed.

There are many possible explanations for these results. Of these, the two most likely appear to be:--

(a) Because the reactions in both mice and goats have been observed only in low passage levels, they may have been due to residual agent which has been carried over at each passage. This is not considered to be the most likely explanation since in calculating the dilution of original inoculum no account was taken of the numerous fluid changes, and the reactions seen in both the goats and mice were atypical. Furthermore, the disease in mice did not appear to progress in animals left for long periods.

(b) The agent of scrapie may have multiplied in the cultures, but that it was modified by passage and no longer produced characteristic lesions in either goats or mice.

I am also indebted to Dr. G. D. Hunter and Mr. G. C. Millson of the Biochemistry Department for permission to refer to their most recent paper which will soon appear in the Journal of General Microbiology. This may be summarised as follows:--

Homogenates of brains from mice clinically affected with scrapie have been fractionated

by differential centrifugation and equilibration in sucrose density gradients. The bulk of the infectivity was found in the heavy particulate fraction containing mitochondria and possibly lysosomes.

Dr. Hunter feels that it is important at this stage to follow only the fate of the bulk of the infective material. Clear-cut results can thereby be obtained. Small amounts of the agent in minor fractions may become of great interest at a later stage, but one's technique needs to be of a very high order of accuracy to distinguish between cross-contamination of fractions and components existing in a special form.

The question as to whether the agent is a large one comparable in size and density with mitochondria and lysosomes or whether it is a smaller agent associated with these large organelles is not answered in this paper. However, more recent work shows that ultrasonic disintegration of the fraction which destroys the integrity of the mitochondria and lysosomes does not affect the scrapie agent. This in itself suggests that the agent is not a very large one. The further finding that, after sonication, the agent is no longer adsorbed by DEAE-cellulose also suggests the release of an agent smaller than the mitochondria. However, the bulk of the agent is still sedimentable after sonication, so that it remains in a particulate form. (Figure 20.)

With the pathological, virological, biochemical and genetical spearheads of attack directed at the causal agent of this enigmatic disease, it is hoped that within a few years it will capitulate and surrender the final pieces of information needed to characterise the agent precisely and shed a revealing light on all the mysteries which still surround the epidemiology of scrapie.

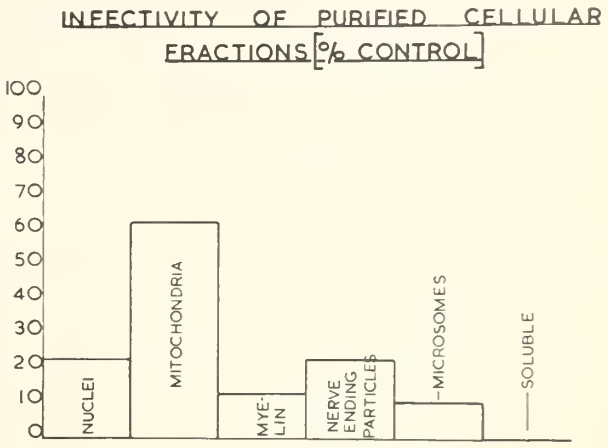


Figure 20.

REFERENCES

(1) Chandler, R. L. (1959) Attempts to demonstrate antibodies in scrapie disease. Vet. Rec., 71, 58.

(2) Chandler, R. L. (1961) Encephalopathy in mice produced with scrapie brain material. Lancet, June 24, 1378.

(3) Chandler, R. L. (1962) Encephalopathy in mice. Lancet, Jan. 13, 107.

(4) Chandler, R. L. (1963) Experimental scrapie in the mouse. Res. Vet. Sci., 4, 276.

(5) Chandler, R. L. (1963) Experimental transmission of scrapie to rats. Lancet, Nov. 30, 1165.

(6) Gordon, W. S. (1959) Symposium on scrapie. Proc. 63rd. Ann. Mtg. U.S. Livestock San. Assocn., p. 286.

(7) Gordon, W. S. & Pattison, I. H. (1957) The experimental production of scrapie in goats. Vet. Rec., 69, 1444.

(8) Hadlow, W. J. (1961) The pathology of experimental scrapie in the dairy goat. Res. Vet. Sci., 2, 289.

(9) Hunter, G. D. (1963) Biochemical investigations on the nature of the agent of 'mouse-scrapie'. J. Gen. Microbiol., 31, xv.

(10) Hunter, G. D. & Millson, G. C. (1963) The comparative infectivities of tissues and of purified cell fractions from mice infected with the scrapie agent. Biochem. J. (in press)

(11) Hunter, G. D., Millson, G. C. & Chandler, R. L. (1963) Observations on the comparative infectivity of cellular fractions derived from homogenates of mouse-scrapie brain. Res. Vet. Sci. (in press)

(12) Hunter, G. D., Millson, G. C. & Meek, G. (1964) The intracellular location of the agent of mouse scrapie. J. Gen. Microbiol. (in press)
(13) Millson, G. C., West, L. C. & Sally M. Dew (1960) Biochemical and haematological observations on the blood and cerebrospinal fluid of clinically healthy and scrapie-affected goats. J. Comp. Path., 70, 194.
(14) Pattison, I. H. (1957) Myopathy in sheep. Lancet, Jan. 12, 104.
(15) Pattison, I. H. (1964) The spread of scrapie by contact between affected and healthy sheep, goats or mice. Vet. Rec., 76, 333.
(16) Pattison, I. H., Gordon, W. S. & Millson, G. C. (1959) Experimental production of scrapie in goats. J. Comp. Path., 69, 300.
(17) Pattison, I. H. & Millson, G. C. (1960) Further observations on the experimental production of scrapie in goats and sheep. J. Comp. Path., 70, 182.
(18) Pattison, I. H. & Millson, G. C. (1961) Scrapie produced experimentally in goats with special reference to the clinical syndrome. J. Comp. Path., 71, 101.
(19) Pattison, I. H. & Millson, G. C. (1961) Experimental transmission of scrapie to goats and sheep by the oral route. J. Comp. Path., 71, 2, 171.
(20) Pattison, I. H. & Millson, G. C. (1961) Further experimental observations on scrapie. J. Comp. Path., 71, 350.
(21) Pattison, I. H. & Millson, G. C. (1962) Distribution of the scrapie agent in the tissues of experimentally inoculated goats. J. Comp. Path., 72, 233.
(22) Pattison, I. H., Millson, G. C. & Smith, K. (1964) An examination of the action of whole blood, blood cells or serum on the goat scrapie agent. Res. Vet. Sci. (in press)
(23) Pattison, I. H. & Sansom, B. F. (1964) Dialysis of the scrapie agent. Res. Vet. Sci. (in press)
(24) Pattison, I. H. & Smith, K. (1963) Histological observations on experimental scrapie in the mouse. Res. Vet. Sci., 4, 269.
(25) Pattison, I. H. & Smith, K. (1963) Experimental scrapie in goats: a modification of incubation period and clinical response following pre-treatment with normal brain. Nature, 200, 1342.
(26) Wilson, D. R., Anderson, R. D. & Smith, W.: 1950. Studies in Scrapie J. Comp. Path., 60, 267.

DISCUSSION

Dr. Goode:

Are there any questions or comments from those present here regarding Dr. Gordon's last presentation?

Dr. Dickinson:

Two questions, both fairly simple and direct.

You previously referred to the fact that Mr. Pattison had passaged further the two strains, you referred to, in goats. Could you amplify that? That is one question. Does sleepy always produce sleepy and does scratchy always produce scratchy scrapie?

Second, what inference must one draw from the situation, which I will also be reporting on later, where you have contact transmission of scrapie in mice, how does this impinge on the kind of experiments you went on to report in studies relating to tissue culture? And, how long did you keep the mice on which you found these small or more extensive pathological changes suggestive of scrapie?

Dr. Gordon:

Mr. Pattison has passaged the scratching and sleepy strains in series through goats for over six generations. The scratching strain has consistently produced the scratching syndrome and the sleepy strain the sleepy syndrome. While it looks as though these two strains have been separated, we feel that each may still be contaminated a little by the other.

I cannot answer your second question until I consult Dr. Haig. Dr. Haig kept the mice inoculated with tissue culture for 18 months, at which time they were discontinued. Those that developed lesions resembling scrapie had

incubation periods ranging from 12 to 18 months.

Dr. Burger:

Dr. Gordon, did anybody in your laboratory determine the sensitivity of the scrapie agent against ether or sodium desoxycholate?

Dr. Gordon:

Dr. Stamp would have that information, and may provide an answer.

Dr. Stamp:

I think it is resistant to both the substances that you mentioned, as far as I know. Certainly it appears to be ether resistant. It is resistant to phenol, methyl alcohol, butyl alcohol, ether; I am sure also resistant to desoxycholate.

Dr. Stuart:

Dr. Gordon, I think the second experiment that you referred to, where rams and ewes were inoculated and then you checked the progeny, did scrapie appear in any of these progeny from rams and ewes in which there were no signs of scrapie?

Dr. Gordon:

It would be better for me to provide a detailed answer to this question later.

In the first experiment 140 ewes and four rams were inoculated with scrapie agent. They mated and produced 112 lambs. Two of the rams were susceptible and two resistant. Forty-five of the ewes were susceptible and 95 resistant. The lambs were not inoculated, yet ten developed scrapie. The sire of these lambs was any one of the four rams. Of the ten lambs that developed scrapie naturally, eight were the progeny of ewes that died of scrapie and two the progeny of ewes that resisted infection.

Further examples appear in Table 2 of the addendum to Paper No. 5 in which it will be seen that two lambs in Group IV that developed scrapie naturally were the progeny of two susceptible ewes by a resistant ram.

Thus offspring of nonsusceptible rams or nonsusceptible ewes may develop scrapie naturally.

Dr. Goode:

Dr. Stuart, does this answer your question, or was your question related to the chronological occurrence of the disease in offspring of ewes that had not shown the disease possibly prior to the time that the lamb showed it.

Dr. Stuart:

That is what I have in mind, whether we have evidence that the disease appeared in progeny from dams in which the disease did not appear. At the same time, it was not known to exist in the sire and dam.

Dr. Gordon:

The parents have been inoculated and this tells us whether these parents are susceptible or otherwise. If the parents resist infection and the offspring take the disease, then this is a case of offspring taking the disease from parents that were resistant to infection.

Dr. Goode:

This seems to be an even more stringent test of what I think was the idea behind your question.

Is that correct?

Dr. Stuart:

Yes, it is.

Dr. Anderson:

This offspring that developed scrapie, was it in contact with other scrapie-infected sheep at any time?

Dr. Gordon:

This is the occurrence of scrapie in offspring in what should be regarded as an infected flock, because the parents have been inoculated with the scrapie agent.

The parents, first of all, are sheep of impeccable origin, that are clear of scrapie. They are inoculated with the scrapie agent and mated. So that this has now to be regarded as an infected flock. The agent has been injected into this flock and if some of the offspring take the disease it would appear to be a natural infection, but the method of transmission from these inoculated parents is still obscure. We don't know how that transmission actually takes place.

Mr. Yohe:

You talked about the inoculation in goats, which seemed to be highly susceptible, and mice.

Do we know of scrapie existing naturally in goats, mice, or for that matter, any other animals.

Dr. Gordon:

I can only speak for the goat. It has been observed in France, and I think at Moredun, they have had a goat which may have been either a natural case or infection by contact.

I don't know which, but not in mice, other than the mice that developed the disease which were in contact with inoculated mice. Those are the only ones I know of that took scrapie without being inoculated.

Mr. Yohe:

As you went through various passages in the mice, do I--perhaps I should have gotten this, but I didn't, thoroughly--do I gather that actually it is being slightly modified so that the effect is somewhat lessened? Or am I misinterpreting this?

Dr. Gordon:

It is quite aggressive and 100 percent lethal in mice when it is being passaged in series. But in the tissue culture material that I was mentioning, there would seem to be a very considerable modification of the agent because it is not producing 100 percent take in mice, but there are a few that develop fairly definite lesions of scrapie. I think it would be Dr. Haig's view that if his tissue culture work means anything at all, the agent he has been able to pass from tissue culture to tissue culture, is considerably modified, because it is not producing 100 percent take and while the lesions resemble scrapie the work is not conclusive of successful propagation of the agent.

Mr. Yohe:

The passing from mouse to mouse at the virulent stage is relatively the same?

Dr. Gordon:

Yes.

Mr. Yohe:

It increases.

Dr. Gordon:

It has a long incubation period in the first transfer from sheep or goats to mice. In the second passage to mice, the incubation period drops to about 5 to 7 months and on continuous passage does not fall below 4 months.

Dr. Mayall:

Dr. Gordon, in one of your slides on goats you said one animal seemed to show signs of scrapie, and then seemed to recover.

Dr. Gordon:

Yes.

Dr. Mayall:

Have you observed this on more than one occasion with goats and have you observed this with sheep?

And I was wondering if you would be able to amplify slightly to say, if you have any information as to the mechanism involved in recovery. I think you mentioned earlier antibody production had seemed to have no part in this. Have you evidence pertaining to this whole field?

Dr. Gordon:

This is a very interesting question which could be elaborated at great length. In brief, we have observed what we believe to be recoveries in both sheep and goats. Sometimes it is difficult in the early stages to determine whether the change from normality is in fact the start of the scrapie syndrome or not--the dividing line is a very difficult one, and one has to keep on examining the animal day after day, until finally convinced that the clinical signs of scrapie are acceptable.

There are occasions when Pattison has warned me that a certain goat was possibly taking scrapie, and we have agreed that the signs were acceptable only to find later that the disease did not become progressive. This has happened when the material inoculated was saliva, and also faeces. So that recovery is by no means unknown.

But you have to remember the diagnosis is subjective and there is not a histological examination in such cases.

Earlier writers, Stockman records five recoveries of very beautifully described cases in sheep. I too have observed recoveries in sheep. And Dr. Stamp has recorded recoveries in sheep.

For example, the five Swaledale sheep I listed in Fig. 7 belonged to a group of six sheep, all showing typical clinical scrapie, five died of the disease while the sixth one had convincing clinical manifestations of scrapie, with a locomotor disorder and had rubbed off much of the wool across its back. It showed every indication that it was as badly affected as its five fellows that died of scrapie.

It gradually began to recover and finally made a complete recovery and we still have this animal alive and have used it to supply serum for various experiments. So I think recovery, though rare, is not unknown.

As to what the mechanism is, this is where I would speculate a bit. In the presence of a number of expert geneticists, I must tread with great caution. On present evidence, I believe that genetic constitution determines susceptibility, and that there are heterozygous resistant, homozygous resistant and homozygous susceptible animals. It is possible that the scrapie agent may survive and be carried by heterozygous resistant animals for long periods, whereas in the homozygous resistant animals, the agent may quite quickly be eliminated while the homozygous susceptible will die if affected.

For the perpetuation and spread of this disease, I am tempted to suggest that heterozygous resistant animals may be the greatest menace in spreading the disease; yet they themselves do not in fact exhibit clinical manifestations. It is in this group that one might expect to find a number of animals that show symptoms and recover.

Dr. Mayall:

This raises another slight point.

Had you ever challenged, rechallenged animals that appear to be resistant to the disease, covering the possibility that for ethnic or biological reasons the first challenge hadn't taken?

Dr. Gordon:

I have done so, on numerous occasions and always found the animals resistant when rechallenged.

The situation is that if an animal is not susceptible, you can inject masses of scrapie material into it on numerous occasions and it remains resistant.

Dr. Dickinson:

I would like to make supplementary comments at this early stage of the discussion on this question which the last speaker from the floor raised, and the first part of the answer. It relates to a question of clinical scrapie.

I think, at this stage, it should be made clear to all who have not worked with scrapie on how difficult it is to diagnose. Scrapie, we have heard, has a 100 percent incidence if you use mice observed to the right age. Yet, unless you are used to observing mice, you can take a mouse at fairly advanced stage, clinically ill, that is to one who is used to observing mice, along to someone who is not, and they say what is wrong with this mouse? They say, "Is anything wrong with it?" Mice at that stage do die, you know. They have lesions, and there is no doubt about it, to someone who knows mice. Ditto in sheep. Ditto in goats.

So we must ram home right away from the clinical point of view it is an extremely difficult disease to diagnose in its early stages. Therefore, one can sometimes be completely wrong on clinical grounds alone.

This is the point at which the Edinburgh workers have found themselves forced to say we define scrapie as a disease which produces a specific neuropathology. It is usually accompanied by clinical signs. This may sound a funny way of putting it.

The situation is such that, unless you are willing to make it clear cut of what you mean by scrapie, and it may not be all-embracing, we cannot talk further because the clinical syndrome is often so vague. Let me give one instance.

Of all the sheep we have had, and most of the sheep on which we have observed scrapie naturally are subjected to neuropathological examination. Out of all those sheep the most severely affected in terms of neuropathology 90 percent of its neurons were severely affected. That particular animal had a team of a dozen of us standing around it to make up our minds and vote on it whether this was a case of scrapie or not.

I don't remember how the vote went. It was an important animal. We had to make up our minds. We wanted to kill it. We had to kill it since we dared not risk the loss of its brain for diagnosis.

The clinical finding in this animal, with this massive damage in its brain, was very, very trivial, indeed. We produced, in fact, something which mislead all of us. We found an animal which had slightly rubbed its wool, nothing more, as though it had just been used to leaning against something, for possibly a reason unrelated to the kind of thing which makes scrapie sheep usually want to rub their wool. There was no wool lost in the immediate sense of the word. And there might have been a slight incoordination.

If you would twist its neck up, it would collapse. And this fact caused us to say it may not be scrapie. We were all proved as wrong as we possibly could be with the hasty diagnosis that we made when we saw the post mortem results.

This was the situation which one is faced with if one wants to talk about clinical scrapie. Therefore, we say we can't. So it may be that you missed something in this recovery situation because of all this.

I don't think, at this stage of scrapie, that anyone can make any constructive comments about possible recoveries, et cetera, et cetera.

Dr. Goode:

Thank you, Dr. Dickinson. This certainly does further elucidate the problem that we are involved with here.

Dr. Berg:

This may have been answered and I didn't catch it. Where there are subsequent animal passages made following these apparent takes in the tissue culture mice--in other words, the mice showed some result--was there brain material subsequently inoculated into further animals, and, if so, what was the result?

Dr. Gordon:

This has been done, but we have to wait a long time, another five or six months before we know the results. There have been sub-inoculations done from the original infected animals, both goats and mice.

Dr. Goode:

And the sub-inoculated animals did come down with the disease?

Dr. Gordon:

We await the result.

Dr. Stamp:

One of the questions which Dr. Gordon was asked about was the question of total resistance and challenge. I think it is wise to point out that the scrapie inoculum that we give to sheep and goats and mice is the raw brain, and I would agree with Dr. Gordon that, given this challenge, then it is extremely difficult to make the animals that resist first challenge go down with a further challenge.

If, however, you dilute this material, then it becomes a different story. If you dilute the brain out to 10^{-5} you give a smaller challenge to some of the animals. Some of the animals will go down, but some remain susceptible to further challenge because the first dose is too small. You have to be careful to distinguish between this and the fact that the challenge we normally give is scrapie brain, which for some reason or other, seems to be the optimum challenge.

It doesn't matter how much brain you put in over and above this, you can't get a higher take. But if you drop the dose down, you can get a lower take.

THE DIAGNOSIS OF SCRAPIE

H. A. McDaniel and L. G. Morehouse [1]
(Presented by H. A. McDaniel)

Introduction and Review of the Literature

Scrapie is an insidious, chronic, degenerative disease of sheep producing lesions in the central nervous system. It is manifested clinically by signs of pruritis, locomotor disturbances, and hypersensitivity or marked dullness. It has been recognized in Europe for over 200 years (1). The first case in North America was reported in Canada (2,3). The first reported case occurred in the United States in 1947 (4). Since that time, it has spread to many parts of the country. In 1952, it was introduced in New Zealand (5). It has recently been reported in India by Zlotnik and Katiyar (6).

The incubation period of experimental scrapie is commonly 4 to 22 months. The most prominent histopathologic lesion is vacuolation of neurons in the brain stem and spinal cord to a degree not approached in any other disease of animals or man.

The etiology of scrapie is still a controversial matter. One group believes natural scrapie is due to a single autosomal recessive gene (7,8,9,10,11,12). However, they concede that it can be artificially transmitted to some sheep, which may not have the correct genetic composition for the natural development of scrapie, as well as to goats and mice. They have adapted the term "provirus" for what they consider to be the genetically determined but artificially transmissible scrapie agent (11).

Other workers, including those at Moredun Institute and Compton Research Station, believe scrapie is a contagious disease due to a self-replicating agent (13,14,15,16). However, neither prolonged boiling nor treatment with ribonuclease or deoxyribonuclease reduces its ability to produce scrapie (17). Experimental oral transmission of scrapie to sheep and goats is reported (18). There is good evidence of natural spread of the disease in sheep and goats (19,20,21).

Sigurdsson and Palsson describe a chronic encephalitis in Icelandic sheep called Rida (22,23). They feel Rida is due to an agent that remains viable on pastures and is capable of infecting sheep for several years. Most workers agree that this disease is scrapie (24). In the same report, they present an interesting hypothesis relating to scrapie and other diseases with a prolonged incubation period and chronic course. They feel the lack of effective antibody production might explain the long incubation period and extended clinical course. Antibodies against scrapie have not yet been demonstrated (25). Changes in serum proteins have not been detected. (26).

Muscular lesions associated with scrapie have been compared with dermatomyositis and muscular dystrophy of humans (7,8). However, subsequent communications report examination of numerous scrapie animals in which muscular lesions were not observed

[1] Pathology Section, Diagnostic Services, National Animal Disease Laboratory, Agricultural Research Service, United States Department of Agriculture, Ames, Iowa.

consistently (27,28,29). One of these workers concluded that scrapie cannot be considered a primary disease of the muscle, and that muscle changes play no apparent part in producing clinical symptoms of that disease. (27).

Vacuolation of neurons is the most spectacular lesion of scrapie. Stamp believes the vacuolated neurons in their great numbers and multiplicity of forms are pathognomic of scrapie (29). Vacuolated neurons do occur to some extent in normal sheep (30,31,32). Numerous studies have been designed to differentiate scrapie affected sheep from normal sheep by differences in both morphologic features and number of vacuolated neurons (33,34,35, 36,38,39,40).

Brownlee found vacuolated neurons in the brains of each of 24 typical cases of scrapie and none in the central nervous system of 64 normal sheep (33). Holman and Pattison examined 75 scrapie sheep and 98 normal sheep (30). They found vacuolated neurons in all of the scrapie animals and in 5 of the normal animals. They concluded that, "clinical symptoms suggestive of scrapie followed by microscopic discovery of vacuolated nerve cells in the medulla oblongata probably constitute sufficient evidence on which to base a diagnosis of the disease." Pattison later reconfirmed this diagnostic criteria and extended the significance of vaculoated neurons to scrapie infected goats (36). The lesions in the brains of mice artificially inoculated with scrapie is similar to the lesions in sheep and goats. (37).

A comprehensive study by Palmer discusses numbers and morphology of vacuolated neurons in both normal and scrapie sheep as compared to those found in infectious diseases, parasitic conditions, trauma, and other diseases of sheep (14).

Zlotnik and Rennie have investigated morphology, numbers and location of vacuolated neurons in normal and scrapie infected sheep. They examined 180 serial sections from the medullas of each of 57 apparently normal sheep of various breeds, mostly cheviots (31). Vacuolated neurons were found in 73.7% of the medullas. As many as 7 vacuolated neurons per microscopic field were found in a very few sheep. The majority of vacuolated neurons in non-scrapie sheep were in the dorsal motor nuclei of the vagus nerve (31,14).

These authors extended their studies to include 60 additional normal sheep, 29 of which were from the so-called scrapie-free area of the United States. Breeds of sheep from the United States included 10 crossbreds, 10 Columbias, 8 Ramboullets, and 1 Hampshire. They concluded that vacuolated neurons can be found in the medulla of apparently normal sheep, including breeds in which natural scrapie has not been reported. However, the number of vacuoles throughout the medulla is very low, and only in exceptional cases exceeds one vacuole per section.

Zlotnik published the results of histopathologic studies and vacuole counts comparing the brain stems of 70 sheep affected with natural scrapie and 70 apparently normal sheep (40). He classified the vacuolated neurons according to the following 6 types: (1) Single vacuoles in healthy neurons, (2) multiple vacuoles, usually small, in normal neurons, (3) single vacuoles in degenerating neurons, (4) many small vacuoles in cell bodies or axons of degenerating cells, (5) multilocular vacuoles in cells undergoing chromatolysis, and (6) large single or multiple vacuoles in necrotic cells. Only the Type 1 vacuolated neurons were found in normal sheep. The presence of the other five were limited to scrapie sheep.

Zlotnik summarized his observations by stating that: "The number of vacuoles present in 54 serial sections from three levels of the medulla of non-scrapie animals did not exceed 100 and were usually much less, while in scrapie animals the corresponding figure was at least 400, and more often, well over 1,000."

The scrapie eradication program in the United States requires slaughter of, and allows indemnity for, all sheep and goats in infected flocks, source flocks, exposed animals moved from these flocks, and their immediate progeny. These eradication measures emphasize the importance of sound diagnostic criteria. The purpose of this paper is to relate our experiences and outline the procedures employed in the diagnosis of scrapie in the Diagnostic Services Section, National Animal Disease Laboratory (NADL). This is the reference laboratory for the national scrapie eradication program.

This paper is based on 125 specimens from scrapie-suspicious animals received from January 1, 1959, to July 1, 1963. Many of the specimens were examined histopathologically by laboratories other than NADL. In all instances, the sheep were necropsied at locations away from NADL and the brain specimen submitted by mail from veterinarians of the Animal Disease Eradication Division, State Diagnostic Laboratories, Colleges of Veterinary Medicine, and practitioners. However, on several occasions, one of the authors (HAM) has performed the necropsy and collected specimens for histological study.

SIGNS

The case histories submitted with the 125 brain specimens from scrapie suspicious sheep were reviewed. Many of these were scanty. Such statements as, "This sheep showed classical signs of scrapie, examine it histologically" appeared too often. However, many of the case histories were thorough and complete. A few clinical manifestations stand out as very meaningful.

Pruritis and locomotor disturbance appear to be the most diagnostic signs. Since scrapie follows such an unusually long incubation period, it is unexpected in animals under 18 months old. In our cases, none were less than 2 years old. Another characteristic of scrapie is its chronic course. In our cases, the duration of signs always exceeded 30 days.

When the case histories are studied individually, it can be seen that pruritis and locomotor disturbances of 30 days or more duration in sheep 2 years or older is an exceedingly good indication of scrapie. Thirty-four positive cases, as opposed to only 1 negative case, fullfilled all these criteria. However, many of the other 24 positive cases probably fit in this category, but the case histories failed to mention at least one of these factors. It is realized that one or more of these manifestations may never be noted in some cases of scrapie. Nevertheless, a clinical diagnosis of scrapie based on pruritis and locomotor disturbances lasting 30 days or longer in sheep 2 years old or older, appears to have an excellent chance of being correct.

The first sign in many cases of scrapie is loss of wool due to the pruritis. The animal rubs and scratches vigorously against any object available, and bites and gnaws at the skin. In addition, hyperexcitability and incoordination, including unusual walking or running movements, slight tremors of the head and neck and rapid muscular tremors of the thighs and flanks, loss of weight, dry lusterless wool, and lagging behind the rest of the flock, may be noted. These manifestations are frequently noticed by the owner or caretaker and hopefully he will call a veterinarian.

The veterinarian should rule out acariasis by examining the affected skin. Other diseases affecting the nervous system that may be confused with scrapie include listeriosis, pregnancy disease, pseudorabies and rabies. Disturbances in locomotion and coordination can usually be detected and evaluated by making the animal walk backward, move to the side, step or jump over obstacles 12 to 18 inches high, and make sharp turns. These exercises are more meaningful if several other sheep are made to perform along with the suspect. Scrapie animals may assume an unusual stance or an abnormal position of the head, such as holding the head in a slightly distended or rotated position, especially when the "scratch reflex" is elicited. This "scratch reflex" is another classical feature of scrapie. It is elicited by briskly rubbing or scratching the animal's back and is manifested by nibbling motion of the lips, rapid viperlike movements of the tongue, and wiggling of the tail stump. However, the "scratch reflex" may infrequently be absent in scrapie or present in other diseases. In all cases, the duration of each sign, as well as the age, sex, breed, source, and registration number if available, should be recorded.

Once the suspicion of scrapie is aroused, State or Federal veterinary regulatory officials should be contacted. Diplomacy by the regulatory veterinarian is needed. The owner must be convinced to let the sheep live until it recovers or death appears certain. (If the sheep recovers, it is presumed the animal did not have scrapie.) Nevertheless, additional inspections should be made from time to time over a period of 6 months due to possible remission of scrapie signs. Scrapie sheep will probably live at least 30 days after manifesting signs. Death usually follows in 2 to 6 months. It is advisable to move the sheep

to a state diagnostic laboratory or school of veterinary medicine where it can be seen at least twice daily and necropsied in the terminal stages of life or within a few hours after death.

Close observation of the suspicious animal and preparations for necropsy cannot be overemphasized. Of the 125 cases, 17 of the laboratory specimens had undergone severe postmortem degeneration, ten had been frozen either before fixation or while in transit, and autolytic changes were prominent in seven.

Before the sheep dies, a source of dry ice or deep freeze facilities, 10% formalin, and adequate instruments to remove the brain and open the spinal canal should be located.

POST-MORTEM EXAMINATION AND LABORATORY PROCEDURES

Diagnostic lesions of scrapie are not discernible grossly and are only apparent in the central nervous system on microscopic examination. Nevertheless, a thorough examination of the entire carcass should be made and the findings recorded.

The instruments and gloves used in removing the brain should be heat sterilized. The frozen part will be used for tissue culture and laboratory animal inoculation.

The brain is removed and processed as follows:

1. Separate the head from the carcass at the atlanto-occipital joint and cut the spinal cord (See figure 1).
2. Remove the skin from the top of the skull and posterior part of nose, including the skin around the eyes.
3. Using a post-mortem, or similar saw, make cut "A" to extend from a point one-half inch posterior to the middle of the orbit to a similar point on the opposite orbit (see Figures 2 and 3). This cut should be about one-half inch deep and slanted in a backward direction.
4. Cut "B" is made on each side of the cranium from the middle of the foramen magnum to a point one inch medial to the orbital rim, cut "A" and cut "B" bisecting at this point. (See Figures 2 and 3.) This cut should be angled about 45 degrees inward.
5. Insert a heavy knife, chisel, or similar object in cut "B", and pry the top of the skull forward. (See Figures 4 and 5.) Precautions should be taken at this step to prevent the attached meninges from disrupting the brain substance, especially the meninges between the cerebral hemispheres and anterior to the cerebellum. Scissors are more suitable than a knife for cutting these membranes
6. Cut the olfactory tract and raise the brain slightly upward so the optic nerves can be cut. (See Figure 6.) As the brain is raised, the pituitary stalk comes into view. The stalk is cut leaving the pituitary gland in its bony fossa.
7. The brain is gently raised upward and backward. By cutting the cranial nerve roots, the brain is freed from the cranial cavity.

NOTE: The removal of a brain intact requires a delicate and exacting technique. The technique as stated here is not the only procedure and will probably be varied somewhat by each individual. The important thing is to get the brain out and shipped with as little contamination, distortion, and laceration as possible.

8. The vertebral arches of the spinal column are then cut away with bone forceps, and the whole of the cord carefully examined for abscesses or other lesions.
9. The cerebral cortex should be removed from the brain stem with a pair of scissors and thumb forceps and frozen immediately. (See Figure 7.) The brain stem should be placed in a container with approximately 2 quarts of 10% formalin. (In no instance should the brain stem be frozen, since it is destined for histopathologic examination.)
10. After the brain tissue has been fixed in formalin 24 hours, it can be immersed in 10% formalin in a 4 to 8 oz. specimen container and forwarded to a laboratory equipped for histopathological

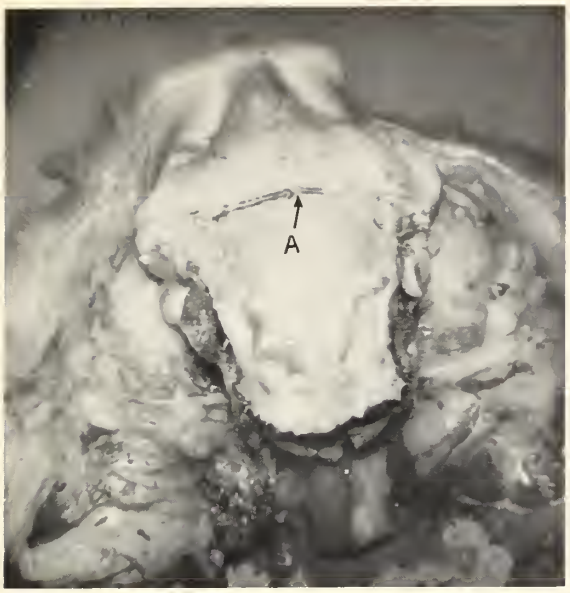


Figure 1.--Note the skin reflected from the midline and cut "A" traversing the forehead.

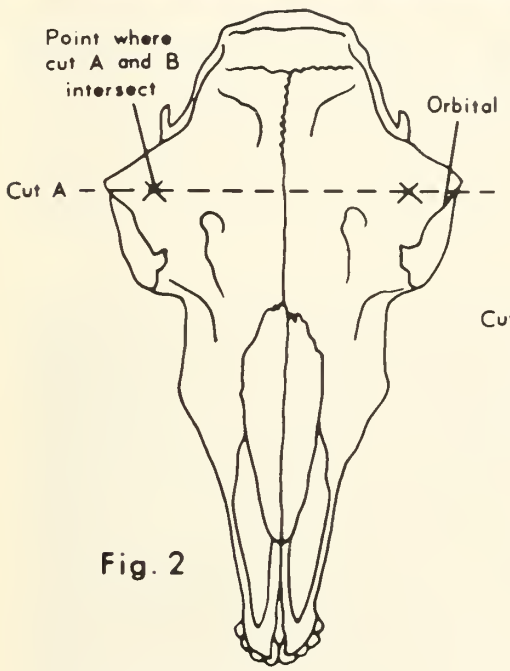


Fig. 2

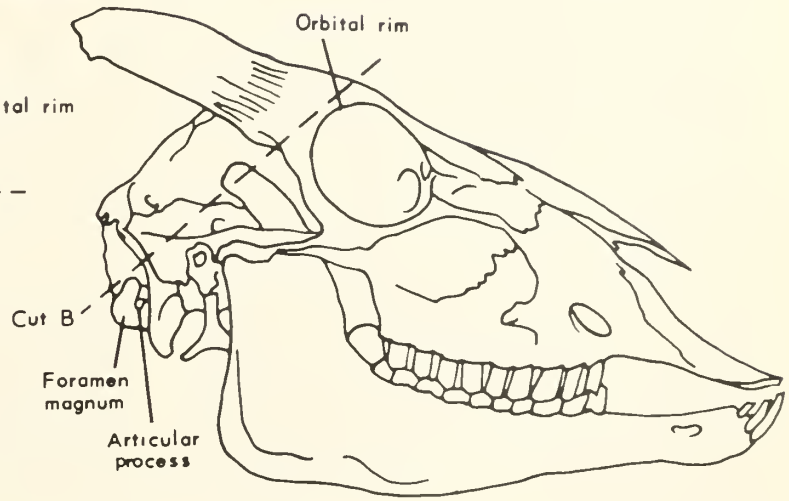


Fig. 3

Figures 2 and 3.--Diagramatic sketches of the two cuts necessary to open the cranium.

examination.* The cerebral cortex packed in dry ice should be forwarded to the same laboratory but since one must remain frozen and the other must not be frozen, it is difficult to pack the 2 specimens together without a shipping carton constructed specifically for this purpose.

The following selection of blocks for histopathological sectioning, based largely on the work of Zlotnik and recommended by Dr. J. T. Stamp,** is

*If the specimens are sent to the Diagnostic Services section of NADL, they should be addressed as follows: Dr. Edward P. Pope, Chief Veterinarian, Diagnostic Services, National Animal Disease Laboratory, P. O. Box 70, Ames, Iowa.

**Personal communication.

Figure 4.--Note cut "B" joining the foramen magnum, and the chisel used to pry off the top of the skull.

Figure 5.--Both cust "A" and "B" are discernible, the "skull cap" is reflected anteriorly. The dura mater has been removed from the surface of the brain.

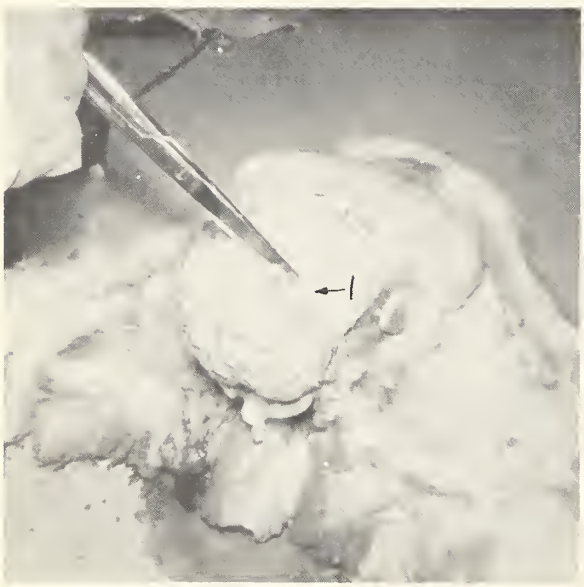


Figure 6.--The optic nerves are being cut (1), the olfactory tracts have been bisected previously. The brain is being reflected dorsally and posteriorly out of the cranium.

Figure 7.--The brain stem and cerebellum on the right (1), are separated from the cerebral cortex (2). The brain stem and cerebellum should be fixed in formalin and the cerebral cortex frozen.

followed where applicable at NADL. (See Figure 8.)

- One block through the thalamus to include the mamillary bodies.
- Two blocks through the mesencephalon to include the red nucleus.
- One block through the pons and cerebellum.
- Three blocks from the medulla (one block to include the trapezoid body, one through the middle, one through the posterior medulla).
- One block of the medulla-cord junction.

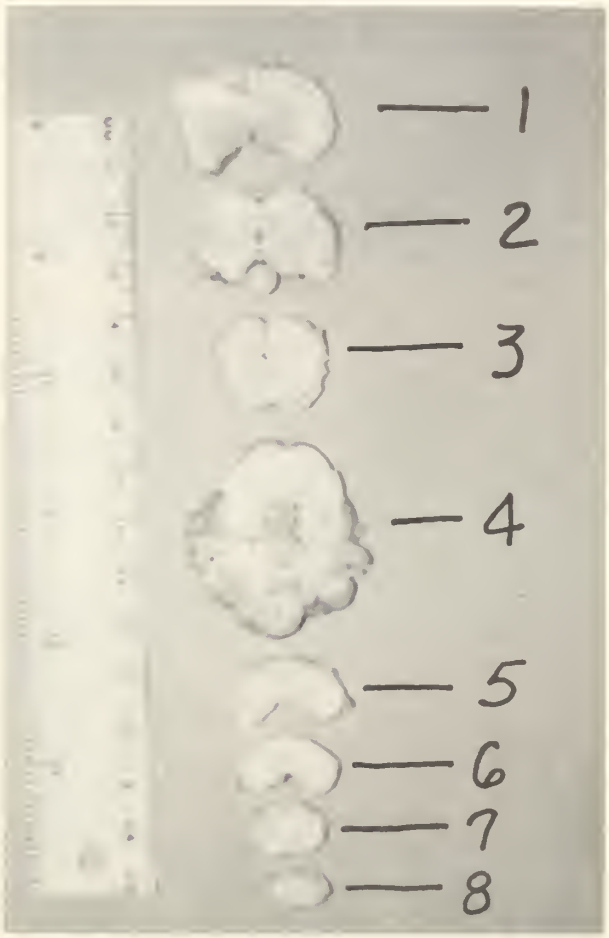


Figure 8.--Tissue blocks cut from the brain stem and cerebellum which are to be dehydrated, cleared and embedded in paraffin. They are from the following: 1. Thalamus including the mamillary body. 2. Mesencephalon including the anterior colliculus and pineal body. 3. Mesencephalon including the inferior colliculus. 4. Pons and cerebellum. 5. Medulla including the trapezoid body. 6. Middle medulla. 7. Posterior medulla. 8. Medulla cord junction.

11. The following sections are mounted, stained and examined microscopically:

Thalamus	- 6 consecutive sections.
Mesencephalon	- 54 serial sections from each block of the mesencephalon. (every third one is examined)
Pons and medulla	- 18 consecutive serial sections from each block.

(A total of 132 sections are mounted for histopathologic examination.)

DIAGNOSIS

Microscopic changes consist of vacuolated neurons, progressive degeneration of neurons, gliosis, and vacuolation of the ground substance (status spongiosa). (See Figures 9 to 14.) The most notable change is neuronal vacuolation, which Stamp suggests is pathognomonic for scrapie. This large "hole" in the cytoplasm appears to result from an outwardly directed force. The Nissl substance, nuclei, and other intracellular components are often pushed to one side of the cell. Frequently the

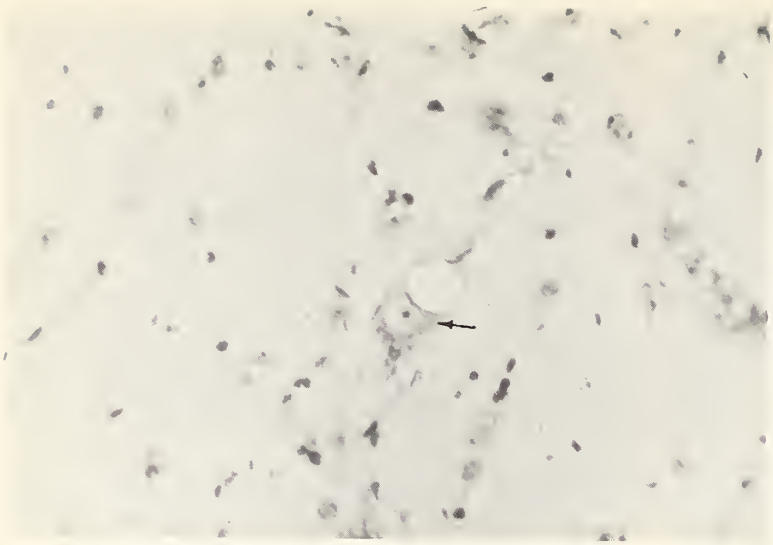


Figure 9.--Single vacuole in a healthy cell classified by Zlotnik as type 1. This is reportedly the only type vacuolated neuron encountered in normal sheep.

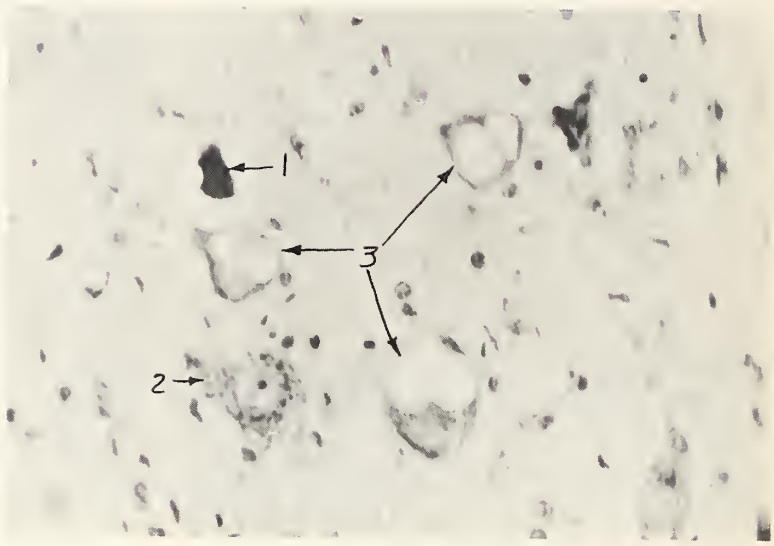


Figure 10.--Scrapie sheep brain: Note pyknotic neurons (1), normal neuron (2), and multiloculated vacuolated neurons (3).

Figure 11.--Scrapie sheep, brain stem, note vacuolated neuron containing intravacuolar material (1); pyknotic neurons (2); multiloculated vacuolated neurons (3); and normal neuron (4).

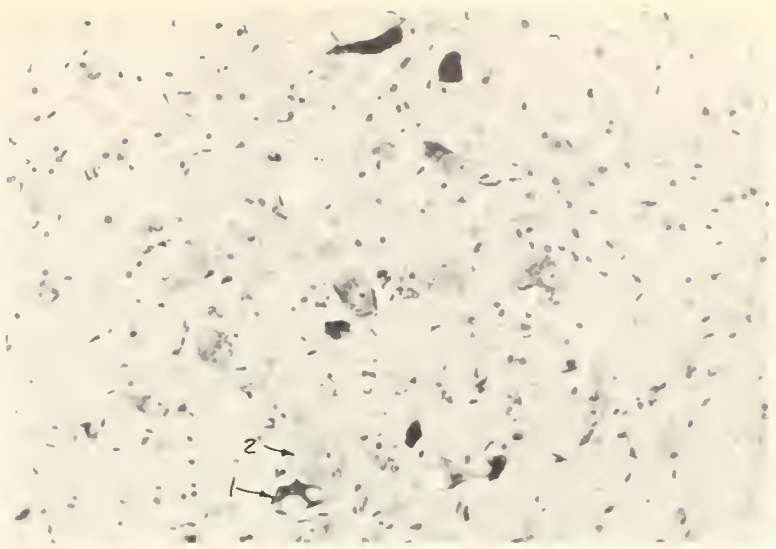


Figure 12.--Scrapie sheep brain stem. Note multivacuolated pyknotic neuron: (1) Adjacent to a normal neuron (2). Other degenerated and normal neurons are observed among prominent microglia and oligodendroglia.

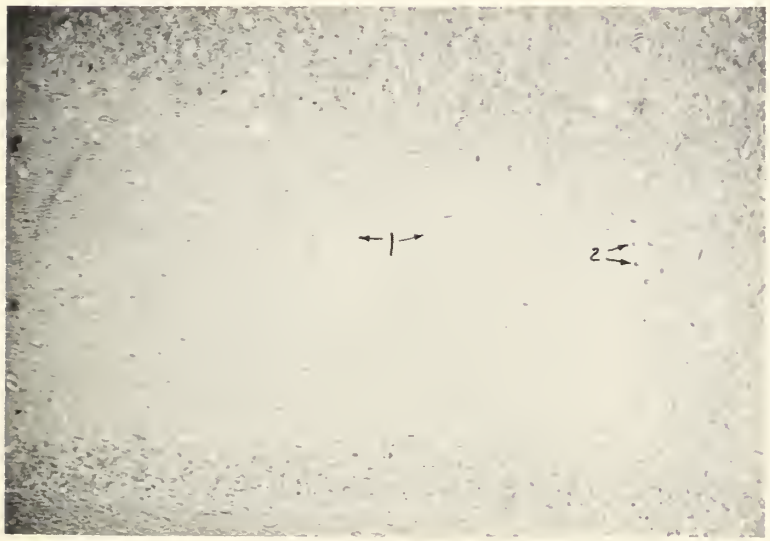


Figure 13.--Scrapie sheep, cerebellum. Note the ground substance vacuolation: (1) In the molecular layer and to a lesser extent in the outer granular layer. Pyknotic Purkinje cells (2) are also discernible.

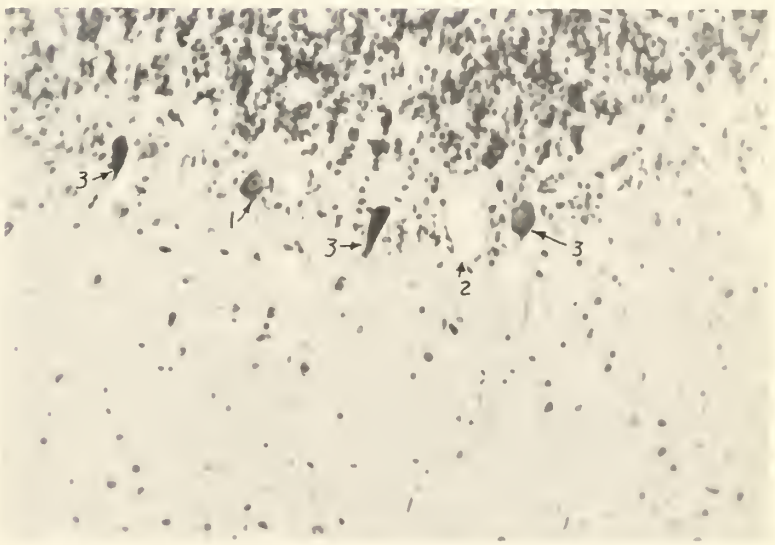


Figure 14.--Scrapie sheep, cerebellum. Note the Purkinje cells: (1) Relatively normal, (2) vacuolated, and (3) pyknotic. Vacuolation is also apparent in the molecular layer.

vacuole is multiloculated and resembles a clump of bubbles. Eosinophilic round bodies may be observed inside the vacuole. We frequently find more than 50 vacuolated neurons per section of medulla in positive scrapie cases. They tend to be distributed in a bilaterally symetrical pattern, i.e., if a nuclear group is severely affected, the corresponding group of neurons on the opposite side of the midline will be similarly affected. In addition, neuronal degeneration characterized by pyknosis, chromatolysis (tigrolysis), sclerosis and other degenerative changes occur in vacuolated and non-vacuolated neurons. (See Figures 10-12.)

The ground substance or brain matrix may also be vacuolated, especially in the cerebellum. (Figures 13 and 14.) It may be diffuse as in figure 13, but is more often focal in groups of neurons which are also vacuolated. Although some of the matrix vacuoles may have resulted from vacuolated neurons in which all remnants of the neuron are missing, this is not always the case. The vacuoles are often more concentrated and numerous than neurons even in normal sheep. Zlotnik thinks edema accounts for many of the acellular vacuoles.

One of the most significant tissue alterations reported to date is gliosis consisting primarily of astrocytes. These cellular changes firmly establish scrapie in its rightful place among the chronic encephalitides. Furthermore, it allows a correlation between scrapie and the human disease called Kuru (41). However, in our opinion, a diagnosis of scrapie can be made without the special staining and impregnation necessary to adequately demonstrate the astrocytosis.

In most cases, it is not necessary to examine the entire 132 sections of brain stem. One or two sections from each block may suffice. Either the history does not indicate scrapie, i.e., reported course of a week or less, or other lesions sufficient to account for death of the sheep are disclosed. For example clinical internal parasitism may result in a chronic course and arouse suspicion of scrapie. The post-mortem examination discloses the parasitism, but the possibility of scrapie may still exist. In such cases, the same procedure is followed except only 2 or 4 sections from each paraffin block are mounted. Edema is usually the only change observed microscopically in the brain tissue.

A diagnosis of scrapie should be made when the clinical manifestations and duration of the disease indicate scrapie, and when vacuolation and degeneration of neurons in the brain stem is in accord with the findings of Zlotnik and other research workers. If the number of vacuolated neurons is large, i.e., 30 or more per section throughout the brain stem and the total count in 54 sections of medulla will exceed 400, confirmation of the clinical diagnosis of scrapie is possible without examining all the sections. Most of the positive cases presented to NADL are in this category; 50 or more vacuolated neurons per section is the usual finding and over 100 per section is not uncommon. If the number of vacuolated neurons is small or portions of the brain stem show no lesions, the complete examination should be made.

The work of Zlotnik serves as an excellent guideline in these cases (40). He reported the numbers of vacuolated neurons did not exceed 100 in 54 sections of medulla from normal sheep, and that his examinations never disclosed less than 400 vacuolated neurons in 54 sections of medulla from scrapie-affected sheep.

REFERENCES

(1) Stamp, J. T.: Scrapie Disease of Sheep, A review of the contradictory evidence as to the nature of the disease. Vet. Record, 70: 50-55 (1958)

(2) Schofield, Frank W.: A Case of Scrapie in an Imported Ewe. Report Ontario Vet. College, Ontario Dept. Agr. Toronto, (1938): 34-35.

(3) Plumer, P. J. G.: Scrapie - A Disease of Sheep - A review of the literature. Canadian J. Comp. Med., 10, (1946): 49-54.

(4) Thorp, Frank, Jr., Judd, A. W., Grey, M. L., and Sholl, L. B.: Scrapie in Sheep. Mich. State Coll. Veterinarian, 13, 1, (1952): 36-37.

(5) Brash, A. G.: Scrapie in Imported Sheep in New Zealand. New Zealand Vet. J., 1, (1952): 27-30.
(6) Zlotnik, I. and Katiyar, R. D.: The Occurrence of Scrapie Disease in Sheep of the Remote Himalayan Foothills. Vet. Record, 73, (1961): 543-544.
(7) Bosanquet, F. D., Daniel, P. M., and Parry, H. B.: Myopathy in Sheep, Its Relationship to Scrapie and to Dermatomyositis and Muscular Dystrophy. Lancet, 271, (1956): 737-746.
(8) Parry, H. B.: Scrapie and Related Myopathies in Sheep. Preliminary Observation on their Investigation and Attempted Control by a Voluntary Health Scheme. Vet. Record, 69, (1957): 43-55.
(9) Beck, E., Daniel, P. M., and Parry, H. B.: Hypothalamic and Cerebellar System Degenerations in Sheep with "Scrapie". Proc. 2nd Int. Cong. Neuropathology, 1961.
(10) Parry, H. B.: Scrapie: A Transmissible Hereditary Disease of Sheep. Nature, 185, (Feb. 13, 1960): 441-443.
(11) Parry, H. B.: Scrapie: A Transmissible and Hereditary Disease of Sheep. Heredity, 17, Part 1, (1962): 75-105.
(12) Draper, G. J., and Parry, H. B.: Scrapie in Sheep: The Hereditary Component in a High Incidence Environment. Nature, 195, (Aug. 18, 1962): 670-672.
(13) Stamp, J. T., Brotherston, J. G., Zlotnik, I., Mackay, J. M. K., and Smith, W.: Further Studies on Scrapie. J. Comp. Path., 69, (1957): 268-280.
(14) Palmer, A. C.: Studies on Scrapie. Vet. Record, 69, (1957): 1318-1324.
(15) MacKay, I. M., Smith, W., and Stamp, J. T.: Experimental Scrapie: Some Recent Work. Vet. Record, 72, (November, 1960): 1002-1006.
(16) Dickinson, A. G., (Reported by Hourrigan, J. L.): Report of Progress and Research on Scrapie. Report of 66th Annual Meeting of the USLSA, (1962): 286-294.
(17) Pattison, J. H. and Millson, G. C.: Further Observations on the Experimental Production of Scrapie in Goats and Sheep. J. Comp. Path. and Therap., 70, 2, (April, 1960): 182-193.
(18) Pattison, J. H., and Millson, G. C.: Experimental Transmission of Scrapie of Goats and Sheep by the Oral Route. J. Comp. Path., 71, (1961): 171-176.
(19) Greig, J. R.: Scrapie in Sheep. J. Comp. Path., 60, (1950): 263-266.
(20) MacKay, J. M. K., and Smith, W.: A Case of Scrapie in an Uninoculated Goat--A Natural Occurrence or a Contact Infection? Vet. Record, 73, (1961): 394-396.
(21) Stamp, J. T., (Reported by Hourrigan, J. L.): Report of Progress and Research on Scrapie. Report of 66th Annual Meeting of the USLSA, (1962): 286-294.
(22) Sigurdsson, Bjorn: Rida, A Chronic Encephalitis of Sheep. British Veterinary Journal, 110, (1954): 341-354.
(23) Palsson, P. Q., and Sigurdsson, Bjorn: Rida, A Slow Progressive Disease Affecting the Central Nervous System of Sheep. Proc. VIII. Nord. Vet. Congr. Helsinki, (1958): 179-191.
(24) Innis, J. R. M., and Saunders, L. Z.: Comparative Neuropathology, Academic Press, New York, (1960): 347-356.
(25) Chandler, R. L.: Attempts to Demonstrate Antibodies in Scrapie Disease. Vet. Record, 71, (Jan., 1959): 58-59.
(26) Avery, R. J., Mills, J. A., and Darcel, C. B. Q.: A Note on Serum Protein in Normal and Scrapie Infected Sheep. Canadian J. Comp. Med. and Vet. Science, 24, (August, 1960): 241-242.
(27) Hulland, T. J.: The Skeletal Muscle of Sheep Affected with Scrapie. J. Comp. Path., 68, (1958): 264-274.
(28) Zlotnik, I.: Cerebellar and Midbrain Lesions in Scrapie. Nature, (1960): 185-785.
(29) Stamp, J. T.: Scrapie: A Transmissible Disease of Sheep. Vet. Record, 74, (March, 1962): 357-362.
(30) Holman, H. H., and Pattison, I. H.: Further Evidence on the Significance of Vacuolated Nerve Cells in the Medulla Oblongata of Sheep Affected with Scrapie. J. Comp. Path. and Therap., 53, (1943): 231-236.

(31) Zlotnik, I., and Rennie, J. C.: The Occurrence of Vacuolated Neurons and Vascular Lesions in the Medullas of Apparently Healthy Sheep. J. Comp. Path., 67, (1957): 30-36.
(32) Zlotnik, I., and Rennie, J. C.: A Comparative Study of the Incidence of Vacuolated Neurons in the Medulla from Apparently Healthy Sheep of Various Breeds. J. Comp. Path. and Therap., 68, (October, 1958): 411-415.
(33) Brownlee, A.: Histopathological Studies of Scrapie: An Obscure Disease of Sheep. Vet. Jour., 96, (1940): 254-264.
(34) Palmer, A. C.: Vacuolated Neurons in Sheep Affected with Scrapie. Nature, 179, (1957): 480-481.
(35) Palmer, A. C.: Distribution of Vacuolated Neurons in Brains of Sheep Affected with Scrapie. J. Neuropath. and Exp. Neurol., 19, (1960): 102-110.
(36) Pattison, I. H.: Mycopathy in Sheep. Lancet, 272, (January, 1957): 104-105.
(37) Zlotnik, I., and Rennie, J. C.: The Pathology of the Brain of Mice Inoculated with Tissue from Scrapie Sheep. J. Comp. Path., 72, (1962): 362-365.
(38) Zlotnik, I.: Significance of Vacuolated Neurons in the Medulla of Sheep Affected with Scrapie. Nature, Letters to the Editor, 180, (August, 1957): 393-394.
(39) Zlotnik, I.: The Histopathology of the Brain Stem of Sheep Affected with Experimental Scrapie. J. Comp. Path., 68, (1958): 428-438.
(40) Zlotnik, I.: The Histopathology of the Brain Stem of Sheep Affected with Natural Scrapie. J. Comp. Path., 68, (1958): 148-166.
(41) Hadlow, W. J.: Scrapie and Kuru. Lancet, 2, (1959): 289-290.

DISCUSSION

Dr. Goode:
Are there any questions or comments from the audience at this time regarding Dr. McDaniel's presentation?

Dr. Rea:
Have you taken any of the brains from sheep that have apparently recovered, from the clinical evidence of the disease, and sectioned them?

Dr. McDaniel:
We have not. I know of no case in which the clinical manifestations were actually strongly suggestive of scrapie in which the animal recovered.

Dr. Dickinson:
This we have done, and the answer is negative.

Dr. Rea:
No Pathology.

Dr. Dickinson:
No pathology corresponding to that of scrapie.

VARIATION IN SUSCEPTIBILITY OF SHEEP TO SCRAPIE AND GENETIC IMPLICATIONS

W. S. Gordon [1]

I am beginning to feel a little homesick at the moment. For that reason and to make the scene a little more vivid for you, I want to show you something of the place where the experimentation I will describe is carried out so that you can see the facilities which enable us to be quite positive that we have distinctly separate "clean" and "scrapie contaminated" areas at Compton.

The dark lines show the boundaries of the estate, to which other areas have been added from time to time, giving us our present total of 2,000 acres. This provides plenty of room to separate one lot of animals from another, to breed in isolation animals of particular types and then remove them to another part of the estate for test purposes.

There are three dairy farms, a pig farm (producing 2,000 pigs per annum), a large isolation compound, adjacent to the main laboratories and six isolation buildings at the north end of the estate, near the village of East Ilsley. The land is farmed to maximum advantage for the production of food and animals to satisfy the maw of the experimental monster and we have a flow of cattle, sheep, pigs and goats, coming from normal breeding units, available to enter experiments if required, or to be disposed of commercially.

These pictures show the main laboratory buildings. In the side view, the building on the left is the small animal breeding unit which helps to boost our total animal population to about 30,000 from mice to cattle.

This site plan shows the layout of the main isolation compound with 22 buildings, each capable of taking 24 adult cattle or larger numbers of young cattle, sheep, pigs or goats.

These pictures are included to illustrate our ability to breed sheep in separate buildings and to remove either parents or offspring, according to the experimental plan, to another part of the estate, where they can be grazed while under test for susceptibility to scrapie. Thus, "clean" animals - and only "clean" animals - are or ever have been maintained in these Ilsley isolation buildings at the extreme northern end of the estate, whereas those to be tested for susceptibility to scrapie are removed to pasture remote from these buildings. The picture of the first crop Dorset Down and Herdwick lambs at present under test gives a fair impression of our spacious countryside.

Having seen a little bit of home before starting seriously on the next paper, I feel rather more comfortable. At least I shall know where to go if my views on the genetic aspects of scrapie get me shot down in flames!

To get to the subject proper:

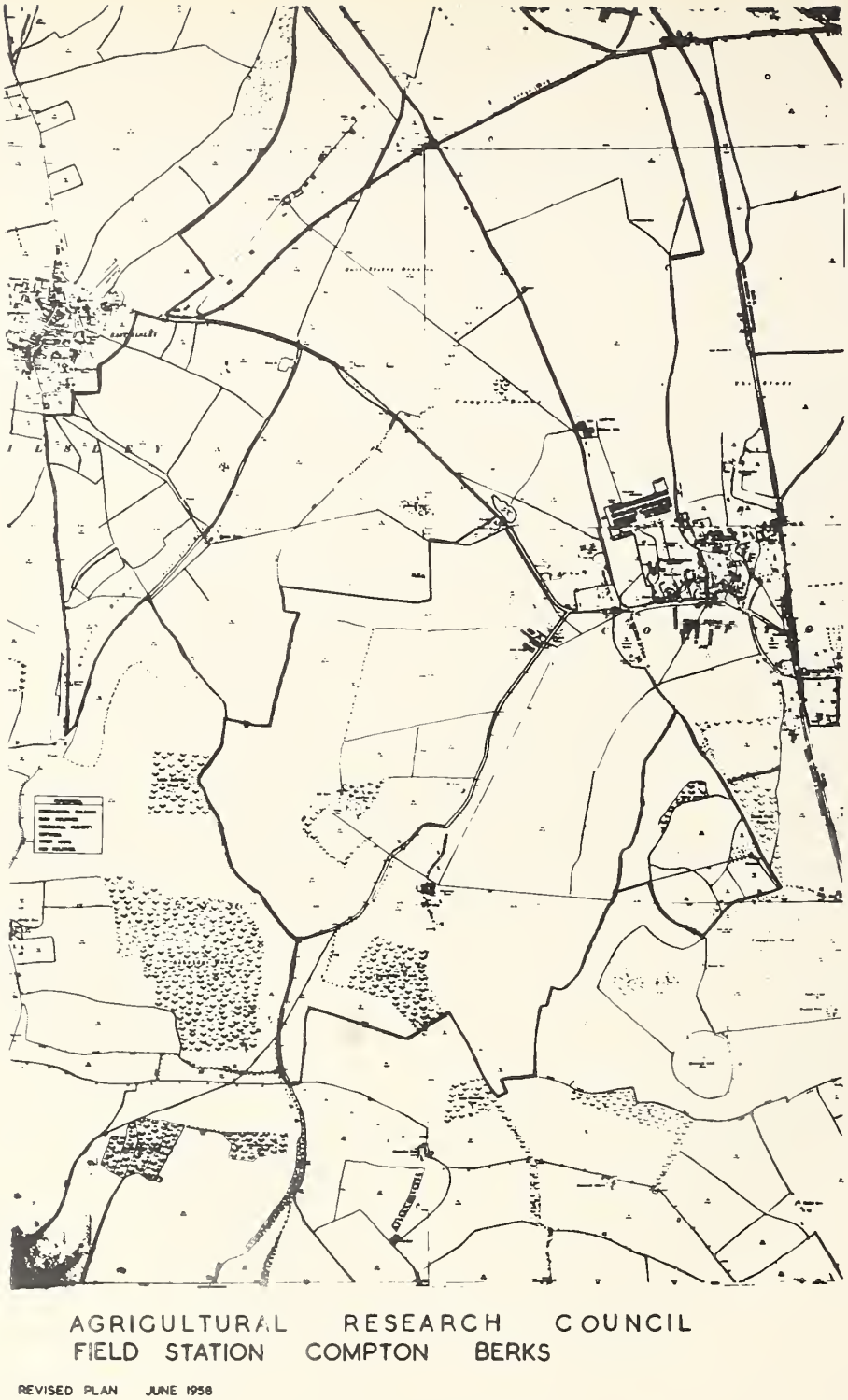
The occurrence of natural scrapie in family lines of sheep has been observed and commented on from the time the disease was first recorded in Britain in the 18th century. The relative importance of genetic factors and infection in the aetiology of this disease remains controversial.

Parry's hypothesis [2] states that naturally occurring scrapie is both hereditary and transmissible by inoculation or other artificial means, but never naturally infectious. It is determined by an autosomal recessive gene s., closely associated with a pro-virus.

Experiments to study the genetic implications of experimental scrapie produced either

[1] Director, Agricultural Research Council Field Station, Compton, Berkshire, England.

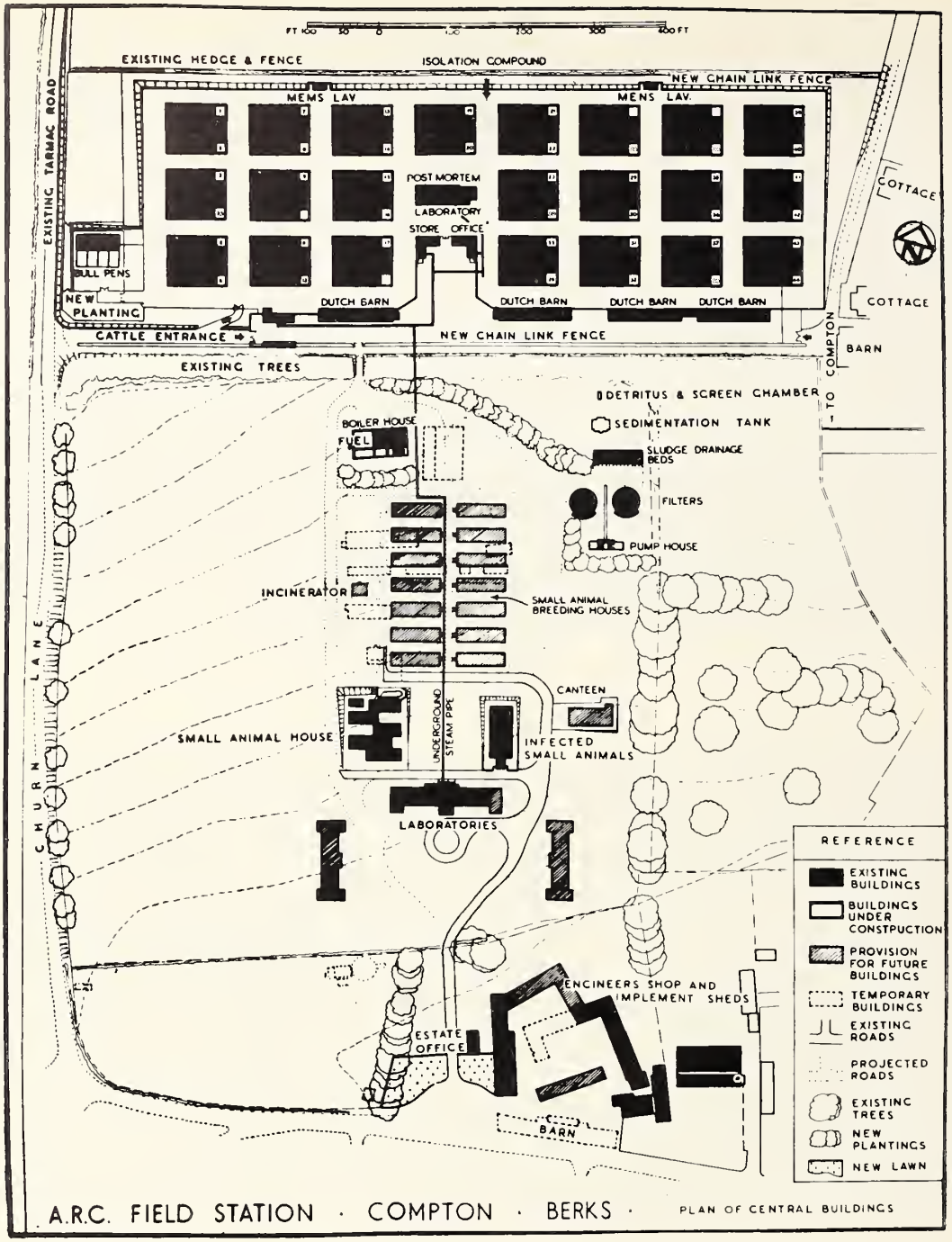
[2] Parry, H.B.: 1962. Heredity, 17: 1, 75.



Plan of the Compton Estate.

Entrance to the Institute for Research on Animal Diseases, Compton

Side View of the Institute.



Plan of isolation compound.

Some of the isolation buildings at Ilsley.

First crop Herdwick and Dorset Down lambs under test for susceptibility.

by inoculation or oral administration were commenced at Compton in 1956.

South Scotland Cheviot sheep from flocks where scrapie had never been known to occur and from which sheep found to be free of scrapie have been purchased for 25 years, were used. They were divided into four groups of approximately 50 ewes, each with one ram. After production of a crop of lambs the sires, dams and offspring identifiable in their family groups were inoculated with scrapie brain material.

Simultaneously, an experiment to determine the comparative susceptibility to experimental scrapie in small sample flocks of 24 different breeds of British sheep was begun.

Each flock was composed of approximately 40 ewes and one ram. These flocks were housed in isolation buildings and when the first crop of lambs was weaned the dams and their male offspring were removed and tested for susceptibility to scrapie by inoculation with scrapie infective brain in the autumn of 1957. Ewe lambs, plus selected ram lambs, were retained in isolation as clean breeding units to produce a subsequent generation.

The original sires were not tested but in the three subsequent generations, as soon as breeding replacements were available, most sires, dams and offspring of both sexes were removed from isolation and tested for susceptibility with scrapie infective material applied by various methods. Thus, the normal breeding flocks were kept completely apart from inoculated animals, in isolation buildings. Separate attendants for affected and normal animals were provided throughout the experiments which terminated in March, 1963.

RESULTS

In the 24 breed experiment the incidence of susceptibility to scrapie within the test period of 24 months post inoculation ranges from nil in the Dorset Down sample to 78 per cent in the Herdwick sample.

Comparison of the overall incidence of scrapie in the descendants with that in the original breed flocks gives very highly significant correlation ($r = 0.667$, $p < 0.001$).

These comparisons are summarised in Fig. 1.

The pedigrees in Figures 2, 3 and 4 illustrate respectively the inheritance of natural scrapie and the inheritance of susceptibility to experimentally induced scrapie in three different breeds of sheep.

INCIDENCE OF SUSCEPTIBILITY TO EXPERIMENTAL SCRAPIE IN SAMPLES OF 24 BREEDS OF SHEEP AND THEIR DESCENDANTS

BREED	ORIGINAL SAMPLE FLOCKS			DESCENDANTS OF SAMPLE FLOCKS			RAMS USED FOR BREEDING		
	NUMBER TESTED	CASES OF SCRAPIE	PER. CENT	NUMBER TESTED	CASES OF SCRAPIE	PER CENT	RESISTANT	SUSCEPTIBLE	PRESUMPTIVE GENOTYPE
HERDWICK	36	28	78	3	1	33	1	1	Rs? rsrs
DALESBRED	43	31	72	27	21	78	1	NONE	Rsrs
SWALEDALE	46	25	54	37	26	70	NONE	2	rsrs? rsrs?
SOUTH SCOTLAND CHEVIOT	45	16	36	32	19	59	NONE	2	rsrs rsrs
DERBYSHIRE GRITSTONE	46	16	35	36	23	64	NONE	2	rsrs? rsrs?
EXMOOR HORN	41	14	34	29	NONE	NONE	2	NONE	Rs?
BORDER LEICESTER	42	11	26	22	8	36	2	NONE	Rs? Rsrs
SCOTTISH BLACKFACE	44	8	18	11	NONE	NONE	1	NONE	Rs?
SOUTH DEVON	35	6	17	21	NONE	NONE	(2)	NOT	TESTED
ROMNEY MARSH	43	7	16	37	1	3	(2)	NOT	TESTED
WELSH CHEVIOT	40	6	15	—	—	—	—	—	—
RYELAND	34	5	15	27	NONE	NONE	2	NONE	Rs?
DORSET HORN	45	6	13	31	NONE	NONE	(2)	NOT	TESTED
SUFFOLK	51	6	12	32	NONE	NONE	2	NONE	Rs?
LEICESTER	42	5	12	33	NONE	NONE	2	NONE	Rs?
WELSH MOUNTAIN	42	4	10	32	15	47	1	1	Rs? rsrs?
HAMPSHIRE DOWN	30	3	10	30	NONE	NONE	1	NONE	Rs?
NORTH SCOTLAND CHEVIOT	45	4	9	50	4	8	2	NONE	Rs? Rsrs
SOUTHDOWN	38	3	8	9	NONE	NONE	2	NONE	Rs?
WILTSHIRE HORN	57	4	7	30	13	43	NONE	1	rsrs
SHROPSHIRE	41	2	5	17	NONE	NONE	2	NONE	Rs?
KERRY HILL	41	1	2	26	NONE	NONE	2	NONE	Rs?
CLUN FOREST	52	1	2	32	NONE	NONE	1	NONE	Rs?
DORSET DOWN	48	NONE	NONE	18	NONE	NONE	1	NONE	RsRs

Figure 1.

Naturally Occurring Scrapie in a Pedigree of Suffolk Sheep

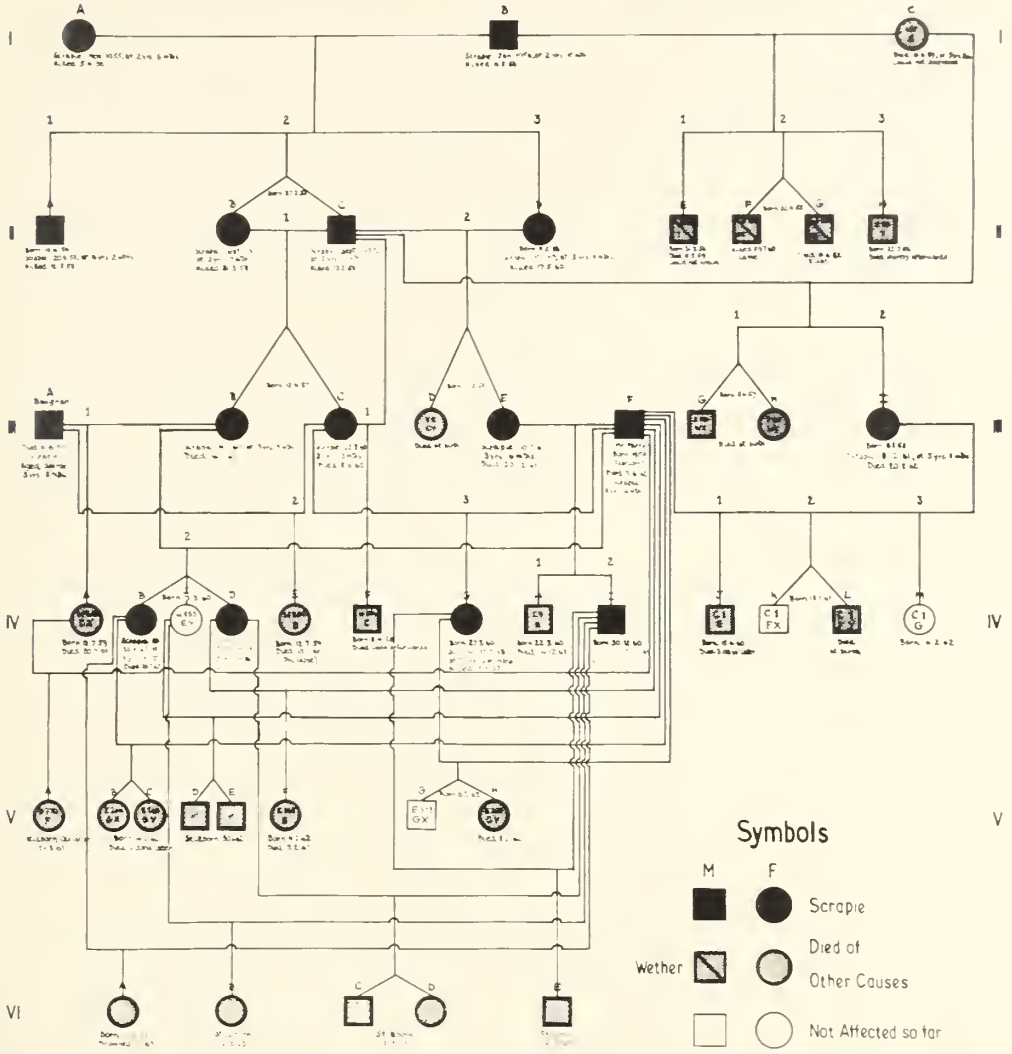


Figure 2.

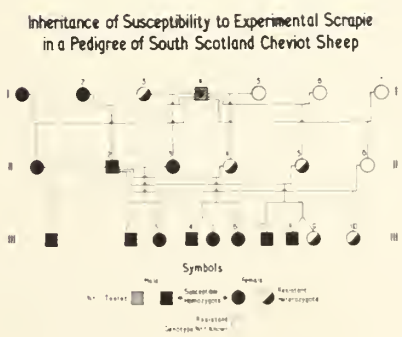


Figure 3.

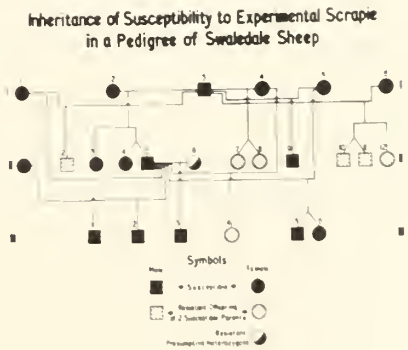


Figure 4.

History of individual sheep in figure 2

LINE I

I A Female 138 S: Scrapie at 32 months
I B Male 136 S : Scrapie at 34 months
I C Female 137 S: Died at 68 months; cause not diagnosed.

LINE II

Offspring of I B x I A:

IIA Male (wether) S 131 T: Born 19.4.54. Scrapie at 50 months
II B - Female Twin S 131 UY)
II C - Male Twins S 131 UX) Born 27.2.55. Scrapie at 31 months
II D - Female S 131 V: Born 11.2.56. Scrapie at 47 months

Offspring of I B x I C:

II E - Male (wether) S 130 T: Born 31.3.54. Died at 62 months, cause not known
II F - Male Twin (wether) S 130 UX)
II G - Male Twin (wether) S 130 UY) Born 22.4.55
S 130 UX killed @ 63 months (lame)
S 130 UY died @ 86 months (bloat)
II H - Male S 130 V: Born 27.7.56, died soon afterwards.

LINE III

III A - Male 118 V, purchased from A. Baughan, 28.8.56:
Scrapie at approximately 40 months.

Offspring of II C x II B:

III B --Female Twin U 60 WX) Born 12.4.57 Scrapie @ 39 months
III C - Female Twin U 60 WY) Scrapie @ 34 months

Offspring of II C x II D:

III D - Female Twin V2 CY) Born 10.2.58 Died at birth
III E - Female Twin Va CX) Scrapie @ 42 months
III F - Male SF 25 K 56, on loan from Mr. Parry: Born February, 1959: Scrapie @ 40 months

Offspring of II C x I C:

III G - Male Twin S 130 WX)
III H - Female Twin S 130 WY) Born 5.4.57, died at birth
III I - Female S 130 C: Born 11.1.58, Scrapie @ 47 months

LINE IV

Offspring of III A x III B:

IV A - Female W 853 DX: Born 12.7.59, died @ 24 months not scrapie, cause not diagnosed

Offspring of III F x III B:

IV B - Female Triplet W 853 EX) Scrapie @ 37 months
IV C - Female Triplet W 853 EY) Born Alive in 1964 13.3.60
IV D - Female Triplet W 853 EZ) Scrapie @ 43 months

Offspring of III A x III C;

IV E - Female W 854 D: Born 12.7.59, died at 18 months prolapse

Offspring of II C x III C:

IV F - Male W 854 C: Born 8.4.58, died soon afterwards

Offspring of III F x III C:

IV G - Female W 854 E: Born 27.3.60 Scrapie @ 39 months

Offspring of III F x III E:

IV H - Male C 9 E: Born 22.3.60, died @ 9 months, cystitis
IV I - Male C 9 EE: Born 30.12.60. Scrapie @ 34 months.

Offspring of III F x III I:

IV J - Male C 1 E: Born 15.4.60, died @ 3 days
IV K - Male Twin C 1 FX) Born 18.1.61 Alive in 1964
IV L - Male Twin C 1 FY) Died at Birth
IV M - Female C 1 G: Born 4.2.62. Alive in 1964

LINE V

Offspring of III F x IV A:

V A - Female D 770 F: Stillborn (by caesarean) 25.3.61

Offspring of III F x IV B:

V B - Female Twin E 266 GX)
V C - Female Twin E 266 GY) Born 14.2.62, died @ 2 days

Offspring of III F x IV G:

V D - Male Twin)
V E - Male Twin) Stillborn 30.1.62

Offspring of III F x IV D:

V F - Female E 268 G: Born 4.2.62, died at 3 days

Offspring of III F x IV G:

V G - Male Twin E 303 GX)	Born 6.2.62	Alive in 1964
V H - Female Twin E 303 GY)		Died @ 2 days

LINE VI

Offspring of IV I x 4 B:

A - Female, born 17.3.63; drowned 7.7.63

Offspring of IV I x 4 C:

B - Female, stillborn 11.3.63

Offspring of IV I x 4 D:

C and D - Male and Female Twins, stillborn 1.3.63

Offspring of IV I x 4 G:

E - Male, stillborn 6.1.63

Figure 2 shows the inheritance of naturally occurring scrapie in a pedigree of Suffolk sheep from high incidence flocks, as determined by Mr. Parry. They were all kept together in a building in the main Isolation Compound at Compton.

Discounting a large number of stillborn lambs and others dying shortly after birth, only one female - I C - and her three male offspring - II E, II F and II G - did not manifest scrapie and so may be presumed heterozygotes. All other individuals, with the exception of IV C, now 46 months old, IV K (36 months), IV M (24 months) and V G (24 months), became affected between the ages of 31 and 50 months with a mean of about 40 months. They are presumed homozygous for the natural scrapie gene. (Parry)

This pedigree supports the hypothesis of autosomal recessive inheritance of natural scrapie with presumptive heterozygotes remaining unaffected. One has to remember, however, that these animals were bred and reared together so that affected and susceptible animals were constantly maintained in close proximity.

Figure 3 illustrates the inheritance of susceptibility to experimental scrapie in a family of South Scotland Cheviot sheep. These were bred in isolation and each of the two succeeding generations were weaned and remained in isolation while the parents were removed and tested for susceptibility.

This family tree and others similar appear to support a hypothesis that genetic factors determine whether or not sheep of scrapie-free origin kept in a scrapie-free environment will become affected with experimentally induced scrapie after challenge by inoculation or oral administration of scrapie infective material. In other words, it would appear from these experiments that, contrary to Parry's hypothesis, the only heritable component in experimental scrapie is a factor determining susceptibility.

This susceptibility can remain undetected for generations unless brought to light by introducing scrapie infective material. The kind of inheritance illustrated in the Cheviot pedigree suggests a simple genetic hypothesis for the inheritance of susceptibility to experimental scrapie. Thus, artificially introduced

scrapie infective agent detects susceptibility in a certain proportion of sheep in each generation. The proportion appears consistent with a single autosomal recessive gene determining susceptibility. The suggested symbol for this gene is rs, while the dominant allele, resistance to experimental scrapie, is designated Rs.

Figure 4 illustrates the inheritance of susceptibility to experimentally induced scrapie in a pedigree of Swaledale sheep, showing a large proportion of resistant offspring resulting from the matings of two susceptible parents.

A considerable number of complete families has now been tested and the result is summarised in Table 1.

Table 1.--Susceptibility to experimental scrapie in the offspring of different parental mating types

Mating type	Offspring			
	Total Number		Resistant	Susceptible
Both parents resistant..... (Note: A large number of these parents may have been homozygous RsRs)	165	Observed	160 (97%)	5 (3%)
		Expected	155 (94%)	10 (6%)
Resistant sire x susceptible dam.....	33	Observed	11 (33%)	22 (67%)
		Expected	16.5 (50%)	16.5 (50%)
Susceptible sire x resistant dam..... (Note: All resistant sires and most resistant dams in these matings presumed heterozygous, Rs rs, on type of progeny)	70	Observed	38 (54%)	32 (46%)
		Expected	35 (50%)	35 (50%)
Totals.....	103	Observed	49 (48%)	54 (52%)
		Expected	51.5 (50%)	51.5 (50%)
Both parents susceptible.....	52	Observed	19 (37%)	33 (63%)
		Expected	None	52 (100%)

Table 1 shows the numbers of susceptible and resistant offspring respectively from the four possible parental mating types. Breeds included are the South Scotland Cheviots used in the 4 Group experiment and 18 of the 24 breeds in the comparative susceptibility experiment.

The proportion of susceptible and resistant offspring of the various parental mating types is in good agreement with a single autosomal recessive gene hypothesis in the following matings:-

PRESUMPTIVE GENOTYPES

Rs Rs x Rs Rs
Rs Rs x Rs rs
Rs rs x Rs rs
} Mating of resistant individuals

Rs rs x rs rs
rs rs x Rs rs
} One parent susceptible

In the matings of two susceptible parents--rs rs x rs rs--there is a large proportion of resistant offspring. This proportion is much in excess of expectation and has been observed in the following breeds:-

Swaledale, South Scotland Cheviot, Welsh Mountain, Derbyshire Gritstone, and Suffolk.

This result throws doubt on the acceptance of the simple recessive hypothesis outlined earlier and suggests that either important modifiers or more than one pair of alleles may be involved.

Some of the simpler suggestions out of many possibles for explaining the occurrence of these exceptions are:-

1. Errors in recording, wrong identification, wrong attribution of offspring to parents. While this might happen quite readily on

farms in other ownership, it is highly unlikely in any large proportion to occur among carefully recorded experimental animals.

2. Errors in diagnosis. Diagnosis was based on clinical signs which became progressively more marked, followed by histological confirmation at death or destruction of the animal. Mild manifestations that did not become progressive were not recorded and so there may have been recoveries that were never diagnosed.
3. Late manifestation. In natural scrapie, Parry estimates about 10 per cent of presumptive ss homozygotes do not manifest the disease by $4\frac{1}{2}$ years of age. In experimental scrapie the incubation period with the inoculum used is 5 - 14 months, with a mean of about 9 months. Most tested resistant animals were disposed of 18 months after inoculation so were given no chance to show "late manifestation", if this existed.
4. Incomplete penetrance. A proportion of presumptive rs rs homozygotes does not manifest the disease at any stage.
5. Incomplete dominance. A number of individuals is actually heterozygote in which a reversal of dominance has taken place so that they exhibit recessive phenotype.

Possible evidence for this last assumption are those matings of susceptibles which produced resistant exceptions and also produced expected susceptible offspring in the proportion of 19 resistant to 8 susceptible, close to 3: 1 ratio expected from mating of Rs rs x Rs rs. Several cases of apparent mild scrapie symptoms with subsequent recovery also provide evidence for a hypothesis that while Rs Rs, the homozygote, is completely resistant, Rs rs is usually resistant but may show a range of manifestation of scrapie from mild symptoms with subsequent recovery to complete susceptibility, expected only in homozygous recessive, rs rs.

WORK IN PROGRESS

The 24 breed experiment demonstrated the remarkable differences in susceptibility to experimental scrapie both between different breeds as well as within them. This susceptibility within breeds can change dramatically according to the genotype of the sire or sires used in any given flock. These observations suggested a possible method of producing two populations whose susceptibility could reasonably be assumed to be diametrically opposed--one 100 per cent susceptible, the other 100 per cent resistant.

Foundation flocks were chosen from the two breeds at either end of the susceptibility spectrum: highly susceptible Herdwicks--incidence in original sample 78 per cent--representing one extreme; highly resistant Dorset Downs--original incidence of susceptibility nil--representing the other. Selective matings, based on classification for susceptibility to experimental scrapie by challenging progeny and parents in turn with scrapie material should result eventually in two populations contrasting greatly, or possibly near 100 per cent, in susceptibility. Such populations would be of immense value for any number of experiments not possible at present because of unpredictable susceptibility in different groups of sheep.

Breeding Programme

Herdwick ewes purchased partly from the same source as the original sample flock and partly from closely related flocks were divided into 43 groups of 22 ewes, each with one ram--a total of 988 Herdwick sheep. Each group was housed separately in specially adapted units at Compton in the autumn of 1961, with shepherds living on the premises.

147 Dorset Down sheep, again partly from the same source and flocks closely related to the original sample flock, were divided into 7 groups of 20 ewes plus one ram and similarly housed in isolation in special units at Compton.

After the first crops of lambs from all groups of both breeds were weaned in the summer of 1962 the lambs were all removed to pastures a considerable distance from the isolation units and inoculated with scrapie brain material in August, 1962. The parents were mated again that autumn to produce a second crop of lambs. The rams were removed in January and inoculated with scrapie material. Following weaning of the second lamb crop in the summer of 1963, all dams were removed to pasture as above and inoculated in August, 1963.

These successive tests should make possible classification for susceptibility to experimental scrapie of the second crop of lambs according to the susceptibility of their sibs and half sibs, challenged first, and that of their sires and dams, challenged second and third. This is assuming, of course, that the mode of inheritance is not very much more complicated than presumed at present. Following this classification of presumptive resistants and susceptibles, matings may be arranged to propagate the presumed strain of susceptibles in the Herdwick breed, while the presumed resistants in this breed are challenged with scrapie infective material. Similarly, a percentage of the presumed resistant Dorset Downs are always drawn off for testing. Eventually, presumed susceptible Herdwicks and presumed resistant Dorset Downs will be crossed and the resulting F_1, F_2, and both backcrosses tested in turn.

RESULTS TO DATE

Of 847 first crop Herdwick lambs challenged in August, 1962, 280 have developed experimental scrapie to date. (confirmed)

41 sires challenged in January, 1963: 6 confirmed cases of scrapie to date, 4 suspected.

832 Herdwick dams challenged in August, 1963: 16 suspected cases to date.

125 Dorset Down first crop lambs challenged in August, 1962: no cases

7 Dorset Down sires challenged in January, 1963: no cases

124 Dorset Down dams challenged in August, 1963: no cases

ADDENDUM

Arising from discussions during and after the Scrapie Seminar, I was requested to provide an addendum to this paper which might be helpful to those deliberating on the future of the scrapie eradication programme.

In the present state of knowledge, the only method of detecting susceptible animals in a flock is by subjecting the flock to a test infection with scrapie infective material. This will eliminate all susceptible animals but probably would have the disadvantage of leaving carriers among the survivors. Table 2 illustrates what can happen when a flock of sheep consisting of six different family groups is challenged by subcutaneous injection with scrapie infective material and it is worth examining in relation to a modified eradication programme in which only the affected and related animals are removed. It shows the distribution of susceptible animals and it will be seen that two of the six sires and some animals in every family group were susceptible. Under natural conditions of infection any one of the susceptible animals might have been the first to show symptoms and it is an interesting exercise to examine each susceptible animal in turn and decide which of its relatives should be eliminated, assuming it was the first clinical case. It will be noted also that two cases of scrapie occurred in the non-inoculated offspring and one manifested clinical scrapie 20 days after inoculation, obviously having been in the final incubative stages when inoculated.

If it is accepted that scrapie can spread by contact at pasture - and from the evidence presented in my previous papers to this Seminar, I believe this possibility cannot be excluded--it has to be remembered that, under conditions of natural infection, it is unlikely that all susceptible animals would become infected at the same time. Consequently, although a whole family is eliminated because one of its members has developed scrapie, and although sheep of remaining families appear normal at that time, no one can say whether any of them has picked up infection. At any time during the succeeding two year period further cases of scrapie may appear among susceptible animals in other families. Experience suggests that this happens with the naturally occurring disease in infected flocks. Consequently, owners who opt for this method of eradication may suffer quarantine for such inordinately long periods that they may be forced eventually to consider the advisability of having the remainder of the flock eliminated.

Table 2 will also serve to illustrate the following points:-

1. Genetic Working Hypothesis

The offspring resulting from the four possible mating types represented in the table appear to fit the simple hypothesis that resistance and susceptibility to experimental

Table 2.--Incidence of scrapie in Cheviot ewes and rams inoculated with Cheviot scrapie brain and their non-inoculated offspring up to the age of approximately 19 months, at which time the surviving offspring were challenged by inoculation with the same material

Parents inoculated 19.11.58 subcutaneously with moredun Cheviot sheep brain pool: rams put with ewes on same day offspring inoculated 8.11.60 subcutaneously with moredun Cheviot sheep brain pool

Group I: Ram C 188: Incubation period 8 months					Group II: Ram C 189: Incubation period 5 months					Group III: Ram C 187				
Ewe	Incubation period	Lamb(s)	Sex	Incubation period	Ewe	Incubation period	Lamb(s)	Sex	Incubation period	Ewe	Incubation period	Lamb(s)	Sex	Incubation period
U 624 VY	8 months	ooo			U 601 V		V 510 DY	M	9 months	U 688 V		(V 502 DX (V 502 DY	F F	
ooo		V 499 D	M		U 668 VY		V 646 D	M	9 months	U 650 V		V 503 DY	F	
U 704 V		V 512 D	M	10 months	U 678 V		(V 563 DX (V 563 DY	M F	9 months	U 666 VY		V 532 D	M	
		(V 522 DX++	M											
U 602 V	9 months	(V 522 DY	F	7 months N.I.	U 696 VY		V 577 D	M	9 months	U 684 VY		(V 562 DX (V 562 DY	M F	
U 634 W		ooo			U 681 V		ooo			U 637 WY		W 806 DX	M	
ooo		V 533 D	M		U 629 W		(W 855 DX (W 855 DY	F F	9 months	U 571 WX		W 846 D	M	
U 601 WY		W 704 D	M	9 months	U 688 WX		W 857 D	M		U 591 W	8 months	ooo		
U 507 W	6 months	ooo			U 652 W	8 months	ooo			U 510 W		(W 868 DX (W 868 DY	M F	7 months
U 568 W	6 months	ooo			U 593 W		ooo			U 690 WY	8 months	ooo		
U 680 WY		W 870 D	M	9 months	U 588 WY		W 898 D	M	9 months	U 639 W		(W 932 DX (W 932 DY	M F	
U 688 WY	8 months	W 858 D	F	9 months	ooo		W 907 D	F	9 months					
					U 566 W	8 months	W 1039 D	M	6 months					

Group IV: Ram U 513 V					Group V: Ram C 190					Group VI: Ram C 186				
Ewe	Incubation period	Lamb(s)	Sex	Incubation period	Ewe	Incubation period	Lamb(s)	Sex	Incubation period	Ewe	Incubation period	Lamb(s)	Sex	Incubation period
U 666 VX		(V 531 DX (V 531 DY	M F		U 684 VX		(V 561 DX (V 561 DY	M F		U 639 V		V 536 D	F	
U 656 V	8 months	V 574 D	F		U 543 VX		ooo			U 652 V		V 597 D	M	
U 521 V		V 602 D	F		U 531 V		ooo			U 555 VX		V 640 D	F	
U 573 V	8 months	ooo			U 550 V	12 months	(V 638 DX (V 638 DY	F F		U 656 D		(W 783 DX (W 783 DY	M M	
U 544 VX	9 months	(V 634 DX (V 634 DY	M	6 months	U 571 WY		W 847 DY	F		U 663 WY	6 months	ooo		
U 661 W	8 months	(W 710 DX +	M	20 days post inoc.	U 511 W		ooo			U 655 WY	6 months	ooo		
		(W 710 DY	F											
U 632 WX	8 months	(W 711 DX	M	12 months N.I.	U 668 WX		W 968 DX	M		U 681 W		ooo		
		(W 711 DY	F	8 months										
U 632 WY	9 months	W 712 D	M		U 668 WY		(W 969 DX (W 969 DY	M M		U 588 WX		ooo		
U 602 W		W 804 D	M		U 538 W		(W 976 DX (W 976 DY	M F		U 518 WY		W 915 D	M	
ooo		(W 805 DX (W 805 DY	M F		U 580 W	8 months	W 1012 D	F						
U 613 WX	8 months	W 861 D	M											

LEGEND

Underlined = Affected with scrapie following inoculation

Framed N.I. = Manifested scrapie without inoculation

W 710 DX + = Manifested scrapie 20 days post inoculation at 18 months of age

ooo = Died of other causes

V 522 DX ++ = Resistant offspring of two susceptible parents

DURATION OF EXPERIMENT

Rams and Ewes disposed of 14th December, 1960 = 25 months post inoculation

Offspring disposed of 10th May, 1962 = 18 months post inoculation

INCIDENCE OF SCRAPIE

Total number of animals involved =149

Died of causes other than scrapie = 21 (14.09%)

Manifested scrapie after inoculation = 37 (24.83%)

Manifested scrapie without inoculation = 3 (2.01%)

Total incidence of scrapie = 40 (26.84%)

scrapie are determined by one pair of autosomal alleles, with resistance completely dominant to susceptibility. On this hypothesis, heterozygous individuals should always be resistant and two susceptible parents should produce only susceptible offspring. There is as yet no single valid explanation for the many resistant exceptions among the offspring of two susceptible parents. (See V 522 DX^{++} in table 2 page 66, table 1 page 63 for the results of the 24-breed and Cheviot 4-group experiments, and page 63 ibid. for suggested explanations of the exceptions).

2. Scrapie Appearing in Non-inoculated Offspring of Inoculated Dams Manifesting the Disease

The two cases of scrapie (V 522 DY, W 711 DX) which occurred without inoculation and W 710 DX, which manifested 20 days after inoculation, in a total of 18 offspring which proved to be genetically susceptible to scrapie on challenge demonstrate that the disease can be transmitted by contact. The mode of contagion is not known. But it seems that dams manifesting the disease are more likely to infect their genetically susceptible offspring than dams which do not themselves manifest scrapie. The sequel to the 4-Group Cheviot experiment described below has some bearing on this point.

3. Naturally Occurring Scrapie in Non-inoculated Offspring Running as a Flock with Their Inoculated Parents

Following the weaning of the first crop of lambs from the 4-Group Cheviot experiment described in Paper No. 5, the four sires, the dams and their lambs were all inoculated at the same time and then run together as an infected flock. The ram lambs were castrated; a second crop of lambs was produced by the four original sires on the original dams and their first-crop daughters. The resulting lambs could not be assigned to their sires. They were all left with the flock for fattening, but were never inoculated. 10 cases of scrapie occurred in this non-inoculated crop, with incubation periods ranging from 7 to 30 months with a mean of about 13 months. Two of the scrapie-affected lambs belonged to dams which had resisted challenge by inoculation, while eight were the offspring of dams which manifested the disease. Of these 8 lambs, 5 had incubation periods between 7 and 9 months. These very short incubation periods may point to a possible infection at birth or even in utero. On the basis of these results, it seems reasonable to assume that the risk of infection in a scrapie-contaminated environment is not negligible for genetically susceptible individuals but that they are more likely to be infected by their own susceptible dams than in any other way (See table 3).

Table 3.--Comparative incidence of scrapie in incoulated and non-inoculated offspring of inoculated dams in an infected flock of South Scotland Cheviot sheep

The same 4 rams--two resistant, two susceptible to scrapie following inoculation--sired both crops of offspring

OFFSPRING

Includes original dams + their first crop daughters	No.	Inoculated 27.11.56				Non-inoculated			
		First crop (born spring 1956)				Second crop + offspring of first crop females (born 1957)			
		Resistant	Susceptible	Total	% susceptible	Unaffected	Developed scrapie	Total	% Affected
Resistant.....	70	42	15	57	26.31	70	2	72	2.78
Susceptible.....	40	12	22	34	64.82	32	8	40	20.00

SEQUENCE OF EVENTS

19.10.55 Rams and ewes first mated
Spring, 1956 First crop of lambs born
7.11.56 Original dams plus first crop daughters mated with same 4 sires
27.11.56 Entire flock inoculated and subsequently run together as an infected flock
Spring, 1957 Second crop lambs born; never inoculated
Autumn, 1958 All surviving 2nd crop male lambs disposed of when 19 months old. Surviving 2nd crop female lambs retained till 2 yrs. 9 months old. One of these developed scrapie at 2 1/2 years old.

Dr. Goode:
Are there any questions or comments regarding this last paper?

Dr. Mayall:
Dr. Gordon, in these experiments what is the source of the inoculum that you used, is it a standard inoculum, and what is the breed of sheep that the inoculum is obtained from?

Dr. Gordon:
The standard inoculum is the scrapie sheep brain pool that we received from Moredun Institute. So we are using exactly the same material that they are using in any tests that they may be performing.

The breed of sheep, I understand, is essentially Cheviot, though there may have been some other breeds incorporated in this at a very early stage in its development.

But it is now constantly being maintained, I think in Cheviot sheep.

Dr. Dickinson:
The last ten passes have been in Cheviots.

Dr. Mayall:
You have had no peculiar results ever using inoculum from a different breed?

Dr. Gordon:
I haven't experienced this. We have used 24 breeds as listed. It matters little whether we take material from a Swaledale, a Cheviot, or any of the breeds listed there.

In testing our multitude of breeds, we always manage to produce cases of scrapie. I know there is difference of opinion at Moredun, where they have certain samples of, I think, Suffolk material which doesn't seem to work quite the same as the Cheviot material.

Mr. Parry:
Are there any natural cases in your pool that--

Dr. Stamp:
Only inoculated brain.

LONG-TERM CELL CULTURES FROM THE BRAIN OF A SHEEP AFFECTED WITH SCRAPIE

D. P. Gustafson, and C. L. Kanitz[1]
(Presented by D. P. Gustafson)

INTRODUCTION

From the standpoint of research progress, perhaps the most limiting characteristic of scrapie has been the remarkably long incubation period in experimental as well as field infections. Transmission of scrapie to mice by Chandler (2) and later by Morris and Gajdusek (5), has been the most auspicious contribution to diminution of the handicap. Hypertrophy and hyperplasia of astrocytes in the encephalon and medulla spinalis of the mouse are similar to such changes described for the goat by Hadlow (4). Bertrand, Carre and Lucam (1) described prominent astrocytosis in naturally occurring scrapie in sheep.

Scrapie became of concern to breeders of purebred Suffolk sheep in Indiana in a relatively brief period following the initial diagnosis late in 1954. Subsequently, in 1958, of 101 grade lambs in the experiment, 24 were inoculated with the supernate of a 10 percent supension of brain from a naturally occurring case of scrapie in a purebred Suffolk ram. Thirty-six to 40 months later 4 of the remaining 10 animals of the group developed scrapie (3). It was from one of the four that cell cultures were prepared from the brain. The morphological differences of the cells and differences in colonial characteristics when compared with similar cultures from an unaffected sheep provided interest in the possible contribution of cell culture to research on the agent of scrapie. Initial observations of the cultures at six months have been reported (3). This report presents amplification of previous work, and further results.

MATERIALS AND METHODS

Cell Cultures

Cell cultures from the brains of sheep were prepared as previously reported (3). Minor variations have been introduced in the composition of the medium. Nystatin is no longer used and calf serum has been substituted for human serum. It was found that fetal calf serum on hand had a deleterious effect and consequently was discontinued in favor of calf serum. The cultures may be maintained in ordinary soft glass prescription flasks, soft glass tubes, or in Leighton tubes of borosilicate. The cell cultures were originally set in Leighton tubes and subsequent serial subcultures were made from individual tubes. Some cultures have been serially transferred more than others. The range is presently 23 to 34 subcultures in 30 months.

Mice White Swiss, and C57BL/6JAX mice obtained from the R. B. Jackson Memorial Laboratory at Bar Harbor, Maine were used in inoculation trials. Inoculations were made intracerebrally in both suckling and weaned mice with 27 guage needle attached to a 1.0 ml tuberculin syringe. Weaned mice were inoculated with approximately 0.03 ml and suckling mice 0.01 ml. Inoculums were cell culture fluids, filtered through an 0.45μ cellulose plastic filter, which had been stored in sealed ampoules at -36° C. Weaned mice were anesthetized with ether prior to inoculation. The site of inoculation was prepared by swabbing with 3.5 percent iodine in 70 percent ethanol.

[1]Department of Microbiology, Pathology and Public Health, School of Veterinary Science and Medicine, Purdue University, Lafayette, Indiana.

MEDIUM TRANSFER TRIALS

Medium from brain cell cultures derived from the sheep affected with scrapie was centrifuged at 10x relative centrifugal force, filtered (03 Selas) and added to similar cultures from a sheep not affected with scrapie. Bubbling pressures of Selas candles were determined and filtration pressures were kept at least 10 lbs. below that during filtration procedures. Observations were made at intervals between 27 and 42 days at which times permanent mounts were prepared. Three serial subcultures were made of such cell cultures over a period of 9 months.

OBSERVATIONS

A. Phase microscopy

Coverslips 11 x 40 mm of #1 thickness covered by growing cells were removed from the Leighton tubes and paraffin mounts were prepared so that photographs could be made of the cells following visualization.

B. Permanent Mounts

Cover slip preparations were stained with either hematoxylin and eosin or using the technique described by Orr et. al. (6) and mounted on glass for permanent record.

RESULTS

A. Cellular Morphology of Living Cells by Phase Photomicrography

Cells from the encephalon of a scrapie affected sheep have been grown for 30 months and subcultured serially as many as 34 times. A comparable culture from an unaffected sheep was lost after 26 months and 28 serial subcultures.

The cells grew singly in the first three weeks of culture gradually increasing in numbers until a cell sheet was formed near the end of that time. Examples of three types of cells present at 12 days are shown in phase contrast photographs in figures 1, 2. and 3. First record of multinucleated cells was made at 15 days of culture (figure 4). At 18 days some cells had large amounts of cytoplasm in contrast to others although nuclear volume appeared to be approximately the same (figure 5).

Gradual accumulation of cells in close proximity to each other occurred about 18 days with multinucleation of increased order and frequency (figures 6 and 7). In the following three weeks to a total of about 40 days the cultures were comprised mostly of the medium sized cells which essentially covered the cover slips in a sheet (figure 8), although the cells with large amounts of cytoplasm (figure 9) were present in significant numbers. By contrast the cells from the unaffected sheep were more uniform in shape and size and the cytoplasm was homogenous (figure 10) and not ribbed as those in figure 8. Furthermore the cells of the unaffected sheep did not tend to overlap one another as they did in cultures from the scrapie sheep. A few of the bizarre forms of the scrapie cultures at 30 days are shown in figure 11 and 12. The propensity of the cells to overlap and for blebbing of the cytoplasm is shown in figures 13 to 16. Heavy granulation of the cytoplasm in many of the cells and not in others in the same field contributed to the unusual wild appearance of the cultures. During this period the nuclear configuration in many cells such as in figure 17 gave rise to the speculation that the nucleus divided by blebbing and constriction rather than by mitosis. The dark, halo-encircled aggregates (figure 18) stained (later) red-violet with May-Grünwald Giemsa stain indicating the presence of DNA. Striations of the cytoplasm of cells as in figure 19 and the greater magnification of the nuclear zone in figure 20 appeared to be ridges as they were progressively accentuated by exposure to white light or to drying. The nuclear bleb observed under phase photography lends credibility to the possibility of such fragmentation as a source of additional small nuclei often seen in the cells derived from the scrapie affected animal. Cells from the unaffected sheep continued to be more uniform in size, more homogenous in cytoplasm, less apt to be multinucleated, and the variation of nuclear size was of a lower order at 49 and 94 days than cells from the

Figure 1. --Scrapie at 11 days. Phase contract. X256.

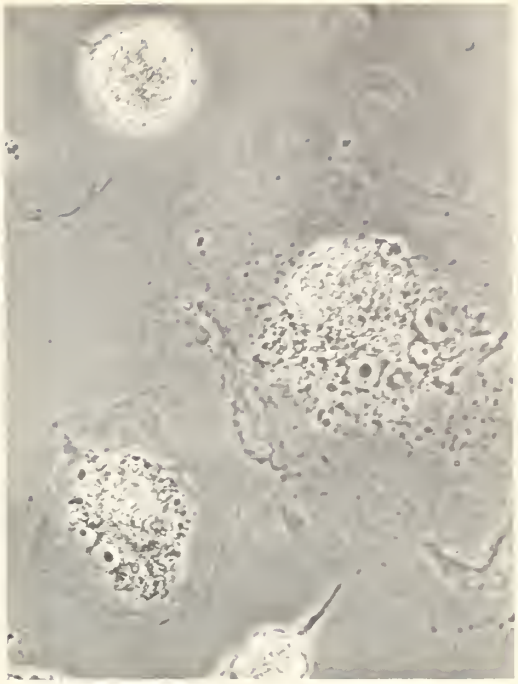


Figure 2. --Scrapie at 12 days. Phase contrast. X256. Note multiple nucleation.

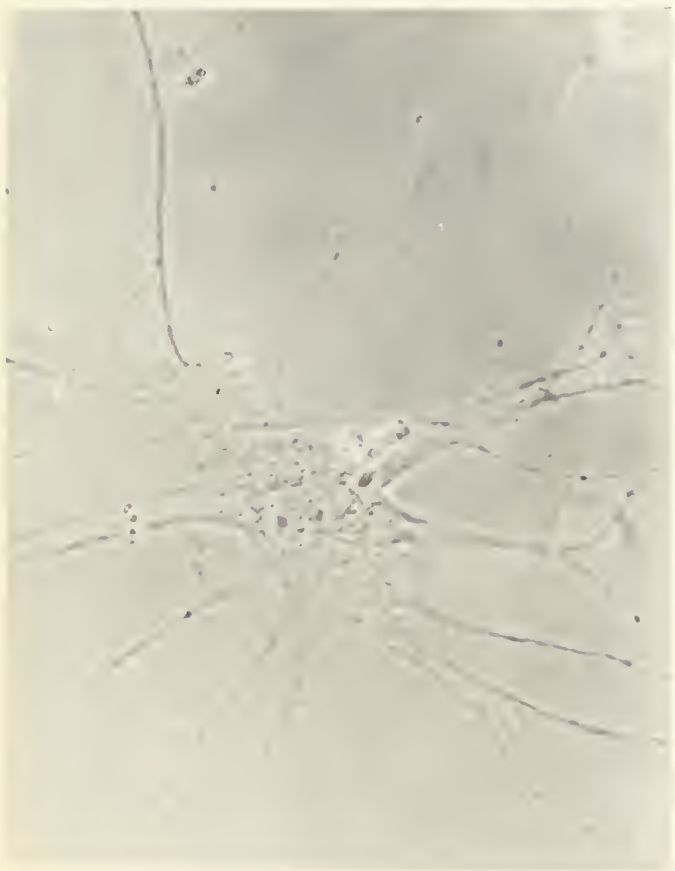


Figure 3. --Scrapie at 12 days. Phase contrast. X256.

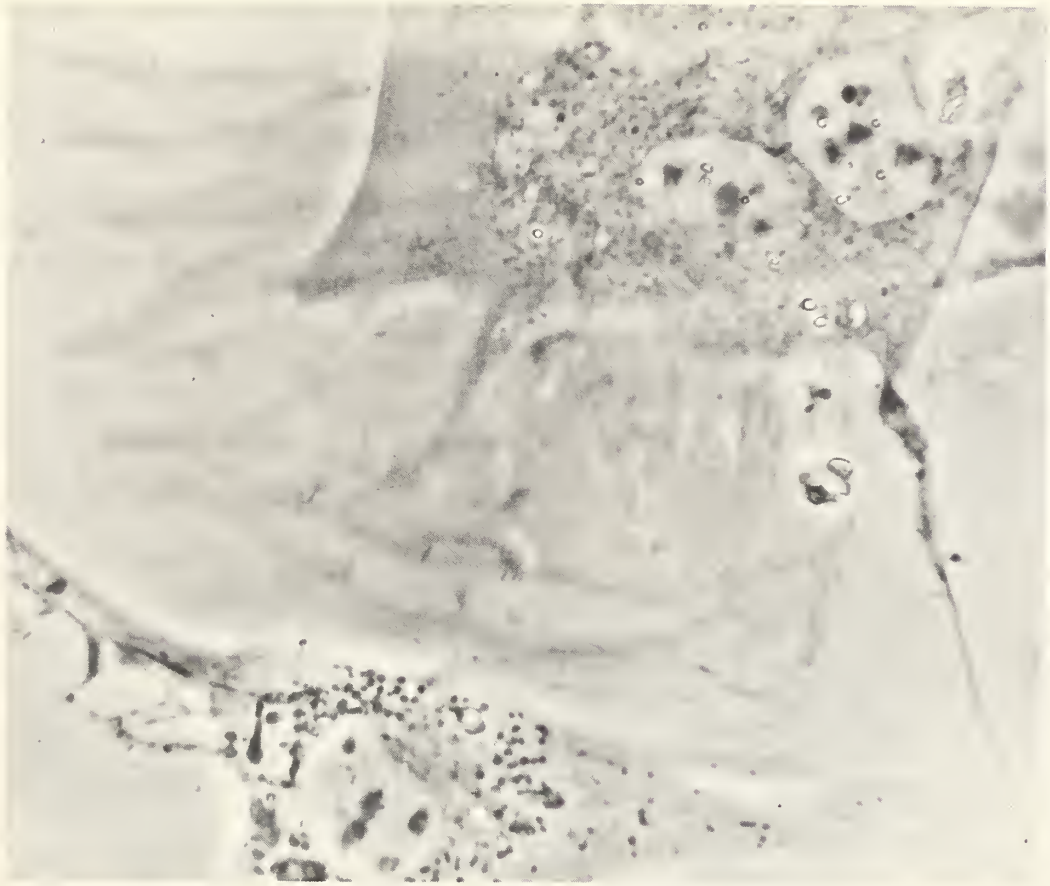


Figure 4. --Scrapie at 15 days. Phase contrast. X800.

Figure 5. --Scrapie at 18 days. Phase contrast. Large amount of cytoplasm and ridging. Cytoplasmic strands with beading characteristic of cells derived from brain. X256.

Figure 6. --Scrapie at 18 days. Nu-nucleus. Phase contrast. X256.

Figure 7. --Scrapie at 18 days. Nuclei are outlined. Phase contrast. X400.

Figure 8. --Scrapie at 30 days. Phase contrast. X320.

Figure 9. --Scrapie at 30 days. Phase contrast. Nuclei are outlined. Remarkable amount of cytoplasm. X320.

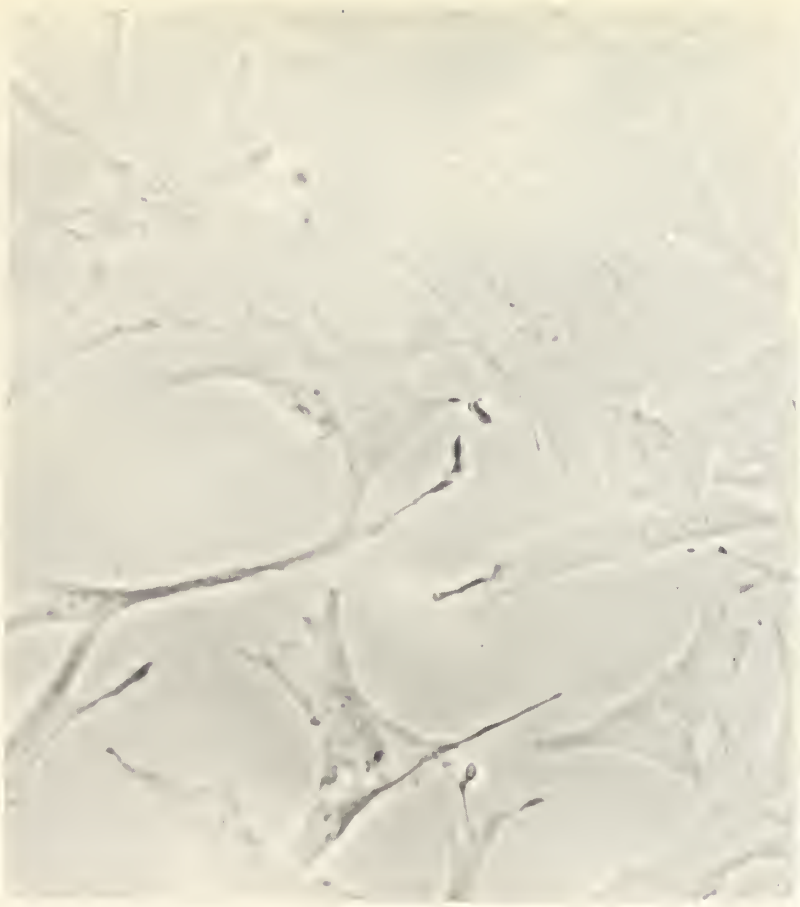


Figure 10. --Normal at 40 days. Phase contrast. Cytoplasm is homogenous, nuclear-cytoplasmic ratio relatively constant, cellular overlapping is minimal. Phase contrast. X320.

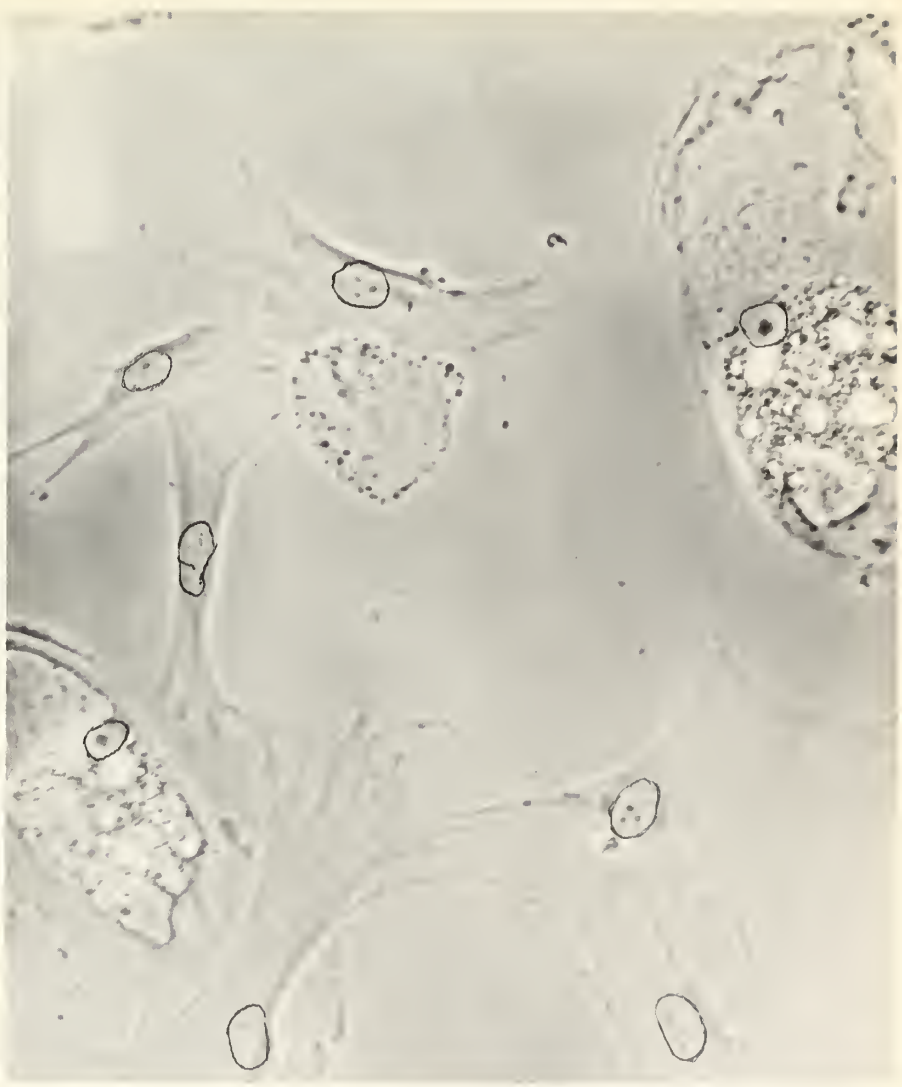


Figure 11. --Scrapie at 31 days. Nuclei are outlined. Phase contrast. X320.

Figure 12.--Scrapie at 31 days. Cell in metaphase. Overlapping of cells. Phase contrast. X320.

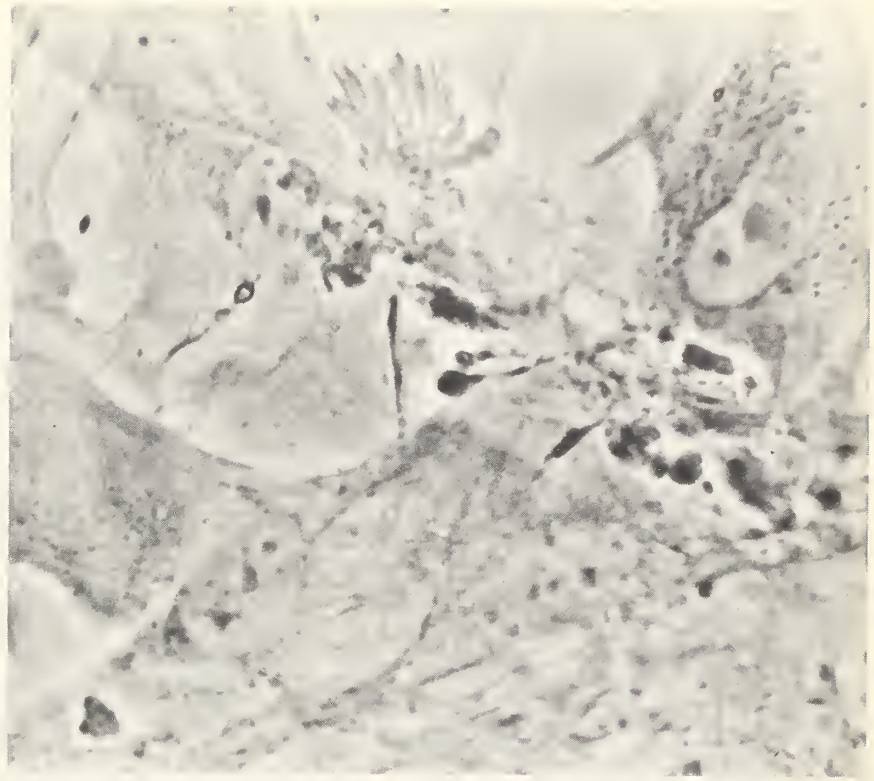


Figure 13.--Scrapie at 31 days. Blebbing of cytoplasm, overlapping of cells, non-polar colonial character. X800.

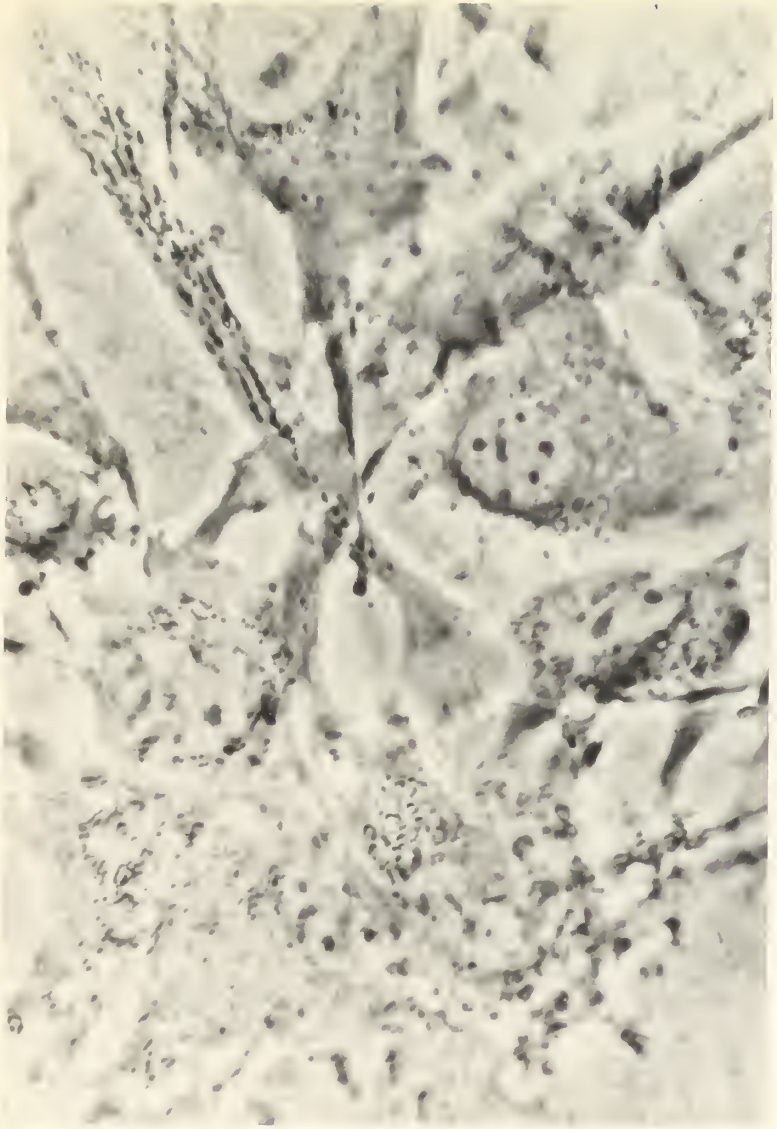


Figure 14. --Scrapie at 31 days. Phase contrast. X800.

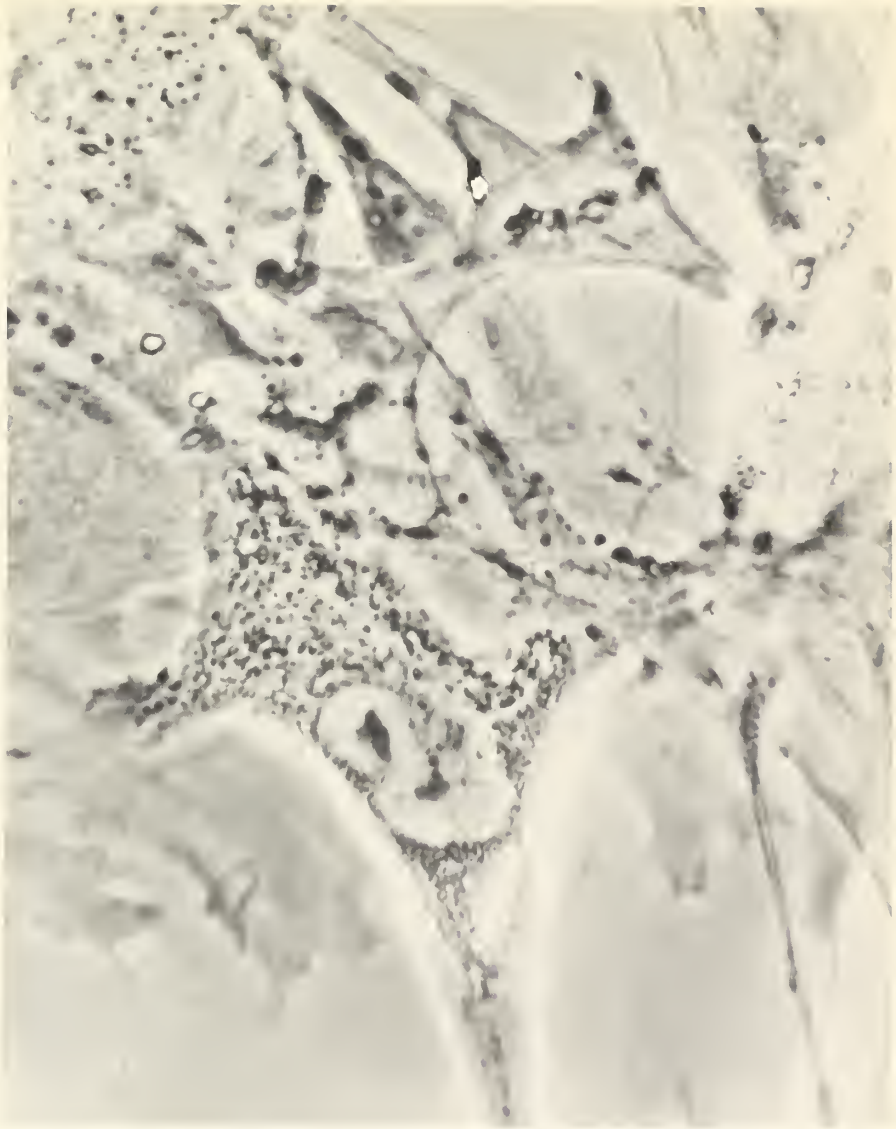


Figure 15. --Scrapie at 31 days. Phase contrast. X800.

Figure 16. --Scrapie at 31 days. Phase contrast. X800.

Figure 17. --Scrapie at 37 days. Multinucleation. Phase contrast. X256.

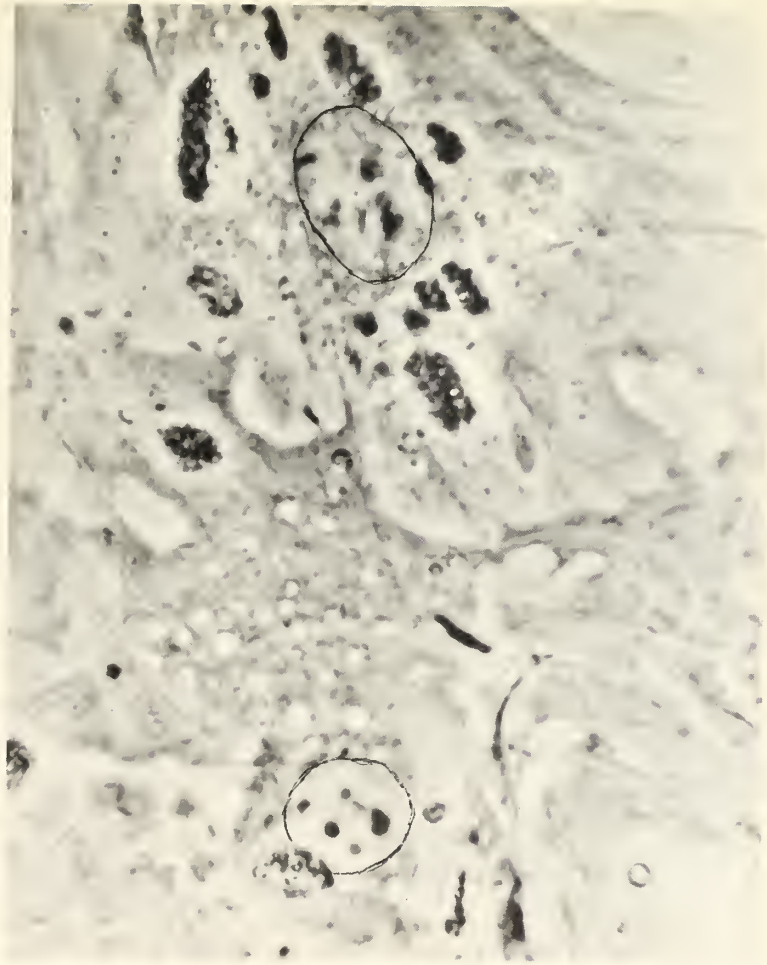


Figure 18. --Scrapie at 39 days. Cytoplasmic aggregates which stain red-violet with May-Grünwald-Giemsa. Nuclei are outlined. Phase contrast. X800.

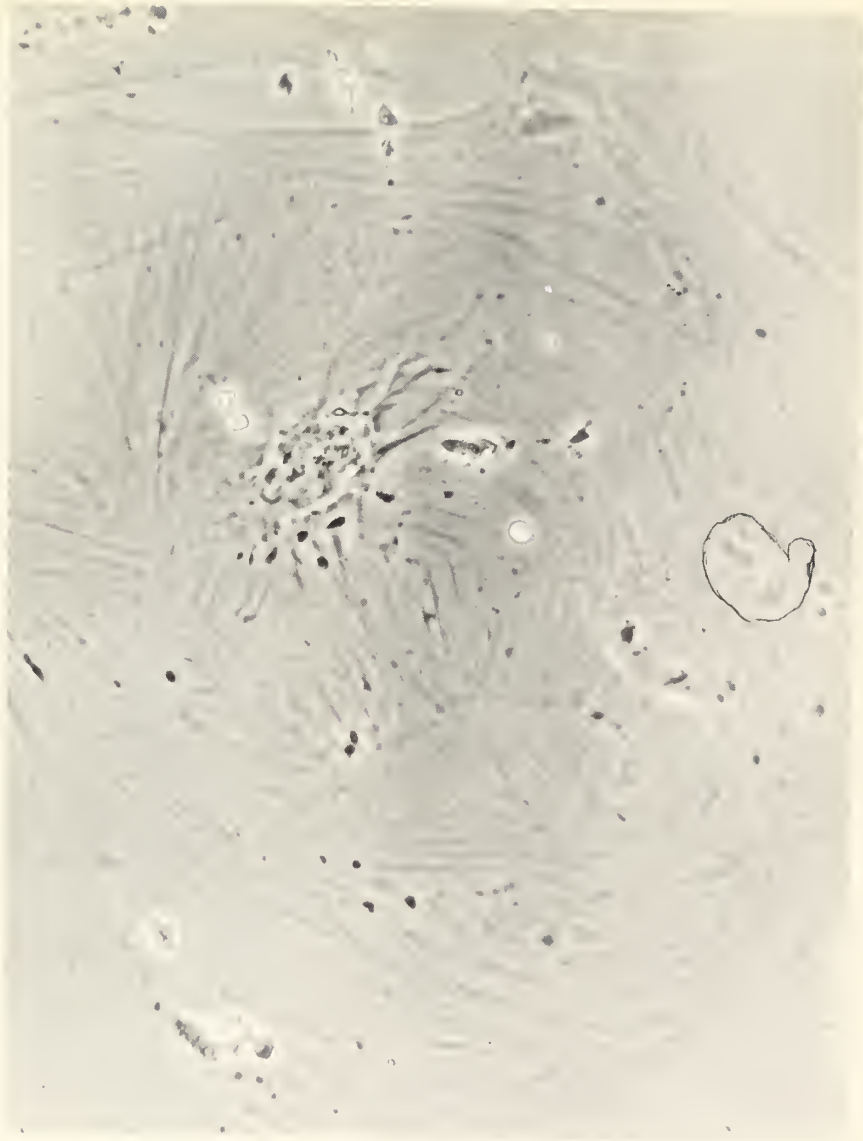


Figure 19. --Scrapie at 39 days. Large cytoplasmic volume with ridging. Nucleus outlined. Phase contrast.

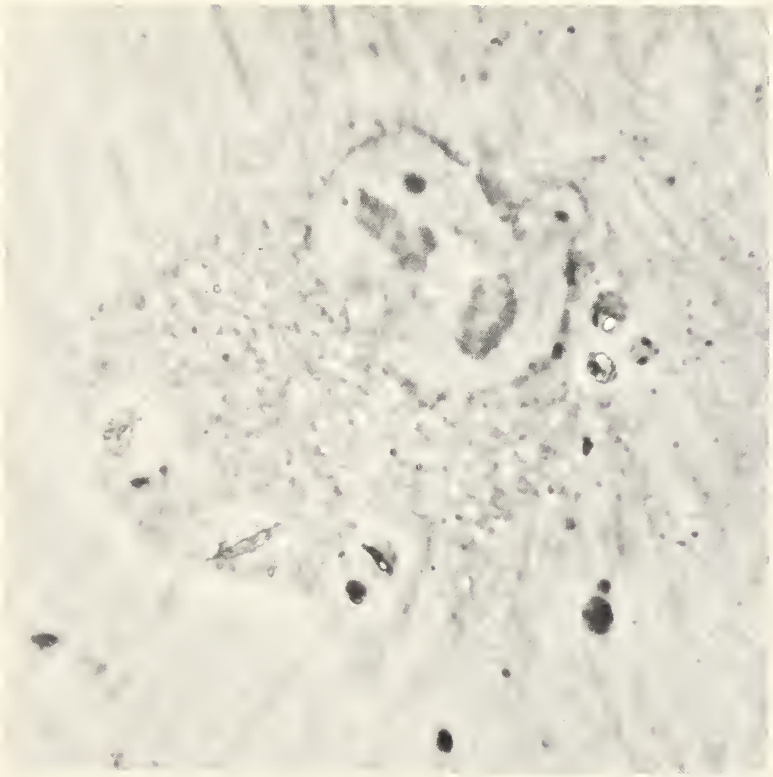


Figure 20. --Higher magnification of nucleus in Figure 19. Phase contrast. X800.

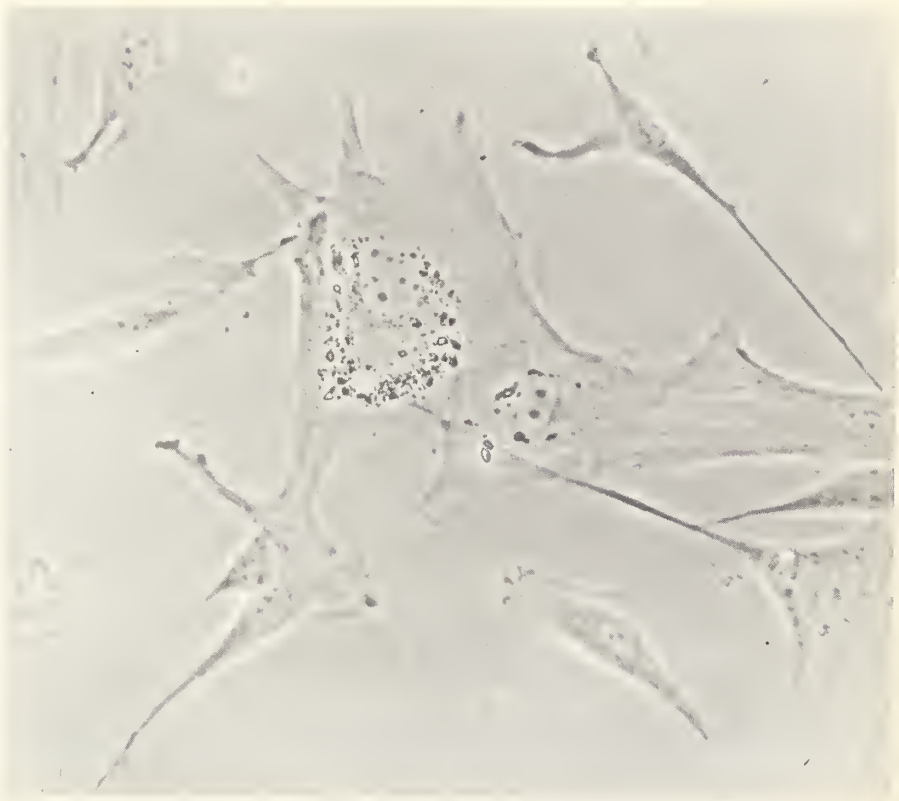


Figure 21. --Normal at 49 days. Phase contrast. X320.

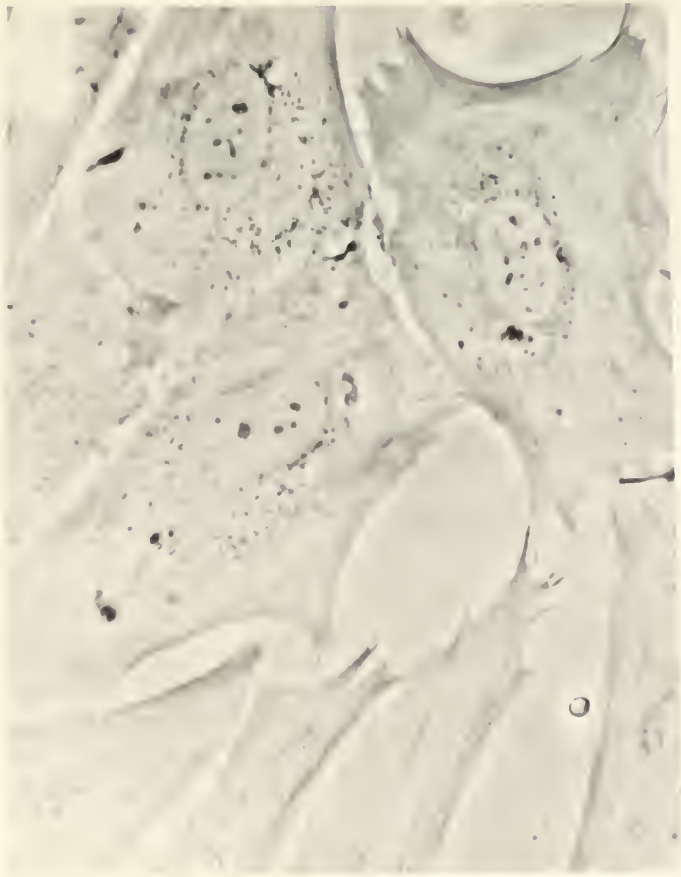


Figure 22. --Normal at 54 days. Phase contrast. X800.

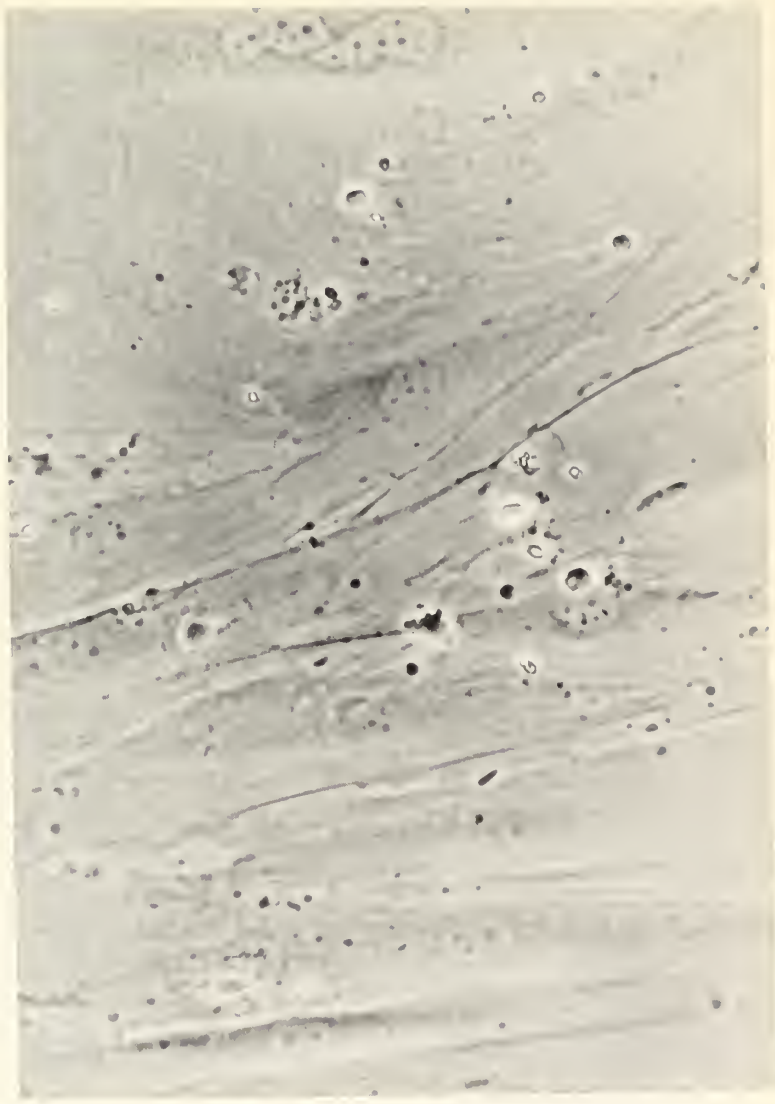


Figure 23. --Scrapie at 94 days. Bizarre nuclear shapes in multinucleated cells. Phase contrast. X320.

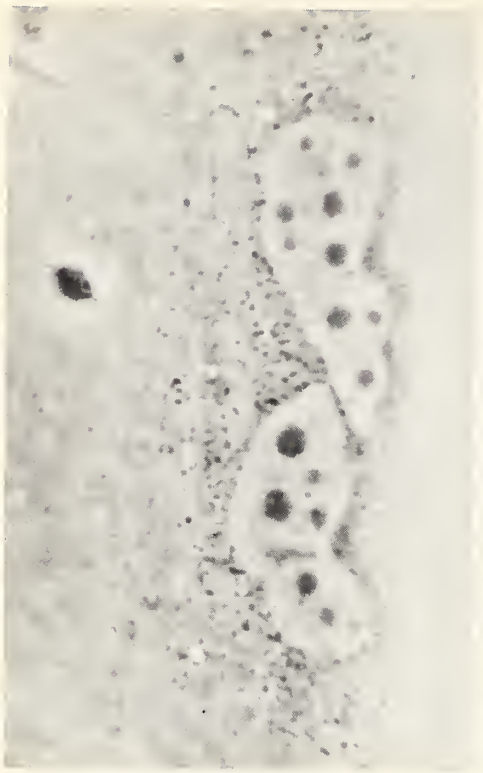


Figure 24. --Nuclei of Figure 23 in higher magnification. Phase contrast. X800.

Figure 25. --Normal at 7 months and serial passage 2. May-Grünwald-Giemsa stain. X10.

affected sheep. (figures 21 and 22) At the same time the characteristics of the cells of the scrapie affected source continued to be as described. (Figures 23 and 24)

B. Cellular Morphology in Stained Preparations

A photograph taken at low magnification, of cell cultures from the unaffected sheep, (figure 25) shows cells of approximately the same size, in whorls of cellular polarity, with minimal piling and overlapping. Phagocytosis of whole cells occurred in both a culture from the unaffected sheep in figure 26 and in the cells from the scrapie sheep in figure 27. Closer inspection of cells from the unaffected sheep at 222 days in culture in figure 28 reveal the homogeneity of the cytoplasm, relatively constant nuclear size, and total nuclear mass. The multiplicity of nucleolar fragments is considered to be related to alcohol fixation preparatory to staining.

Cultures of cells from the scrapie sheep shown in figure 29, 213 days in culture have a remarkable variation in nuclear size and relative cytoplasmic volume; multinucleation is of high order and frequency; cellular polarity is not well maintained and overlapping of cells is common. In sparsely populated areas multinucleation was often well developed and was readily observed as in figure 30. Disparity in size of two nuclei in a single cell is often seen as shown in figure 31. Blebbing and bizarre multinuclear forms are common as shown in figure 32.

Cultures from the unaffected sheep exposed to cell-free filtered medium from cell cultures derived from the scrapie affected sheep were first recorded to show characteristics reminiscent of the latter at 27 days to 42 days after exposure. Overlapping of cells, lack of polarity, wide range of nuclear and cellular size, multinucleation, and blebbing of the nucleus developed as shown in figures 33, 34, 35, and 36.

C. Inoculation of Mice with Cell Culture Medium

Three of six white Swiss mice inoculated at 13 days of age with cell culture medium

Figure 26. --Normal at 145 days and 3 serial passages. Whole cell phagocytosis. May-Grünwald-Giemsa stain. X100.

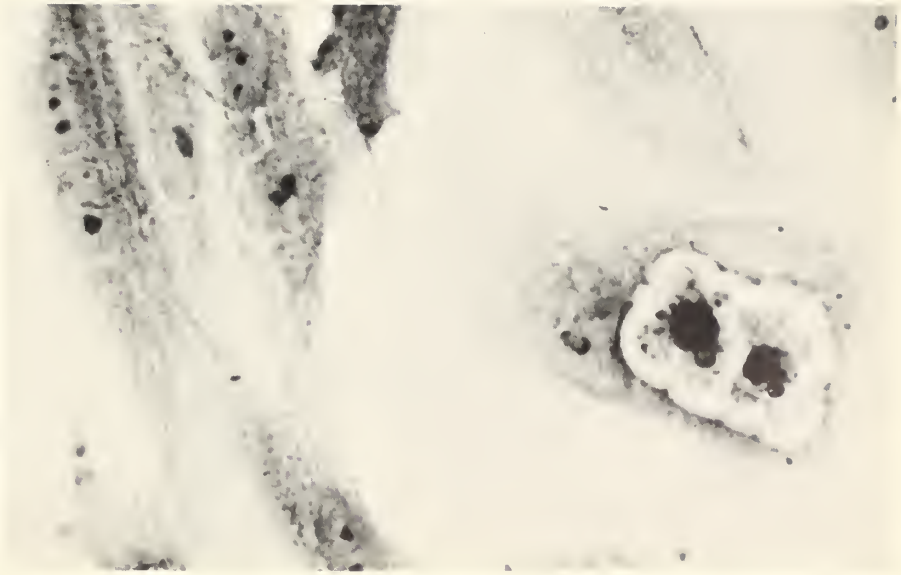


Figure 27. --Scrapie at 152 days. Whole cell phagocytosis and multinucleation. May-Grünwald-Giemsa stain. X160.

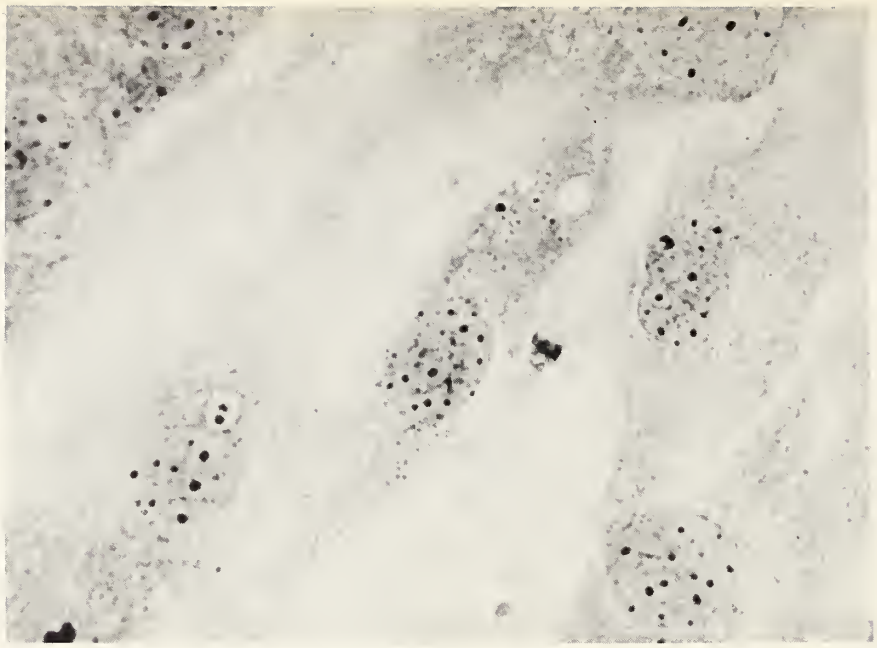


Figure 28. --Normal at 222 days. May-Grünwald-Giemsa stain. X160.

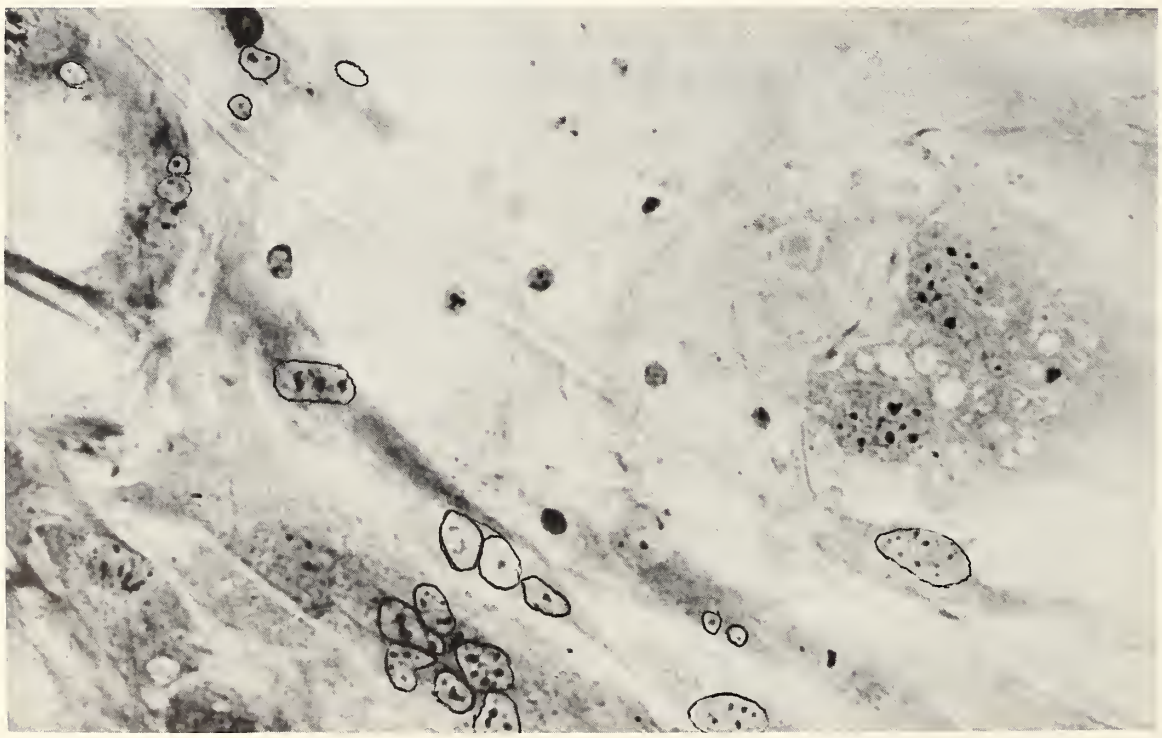


Figure 29. --Scrapie at 243 days and 9 serial passages. Some nuclei are outlined. Wide variation in nuclear size, multinucleation. May-Grünwald-Giemsa stain. X100.

Figure 30. --Scrapie at 151 days. Multinucleation of high order. Nuclei are outlined. Hematoxylin-eosin stain. X40.

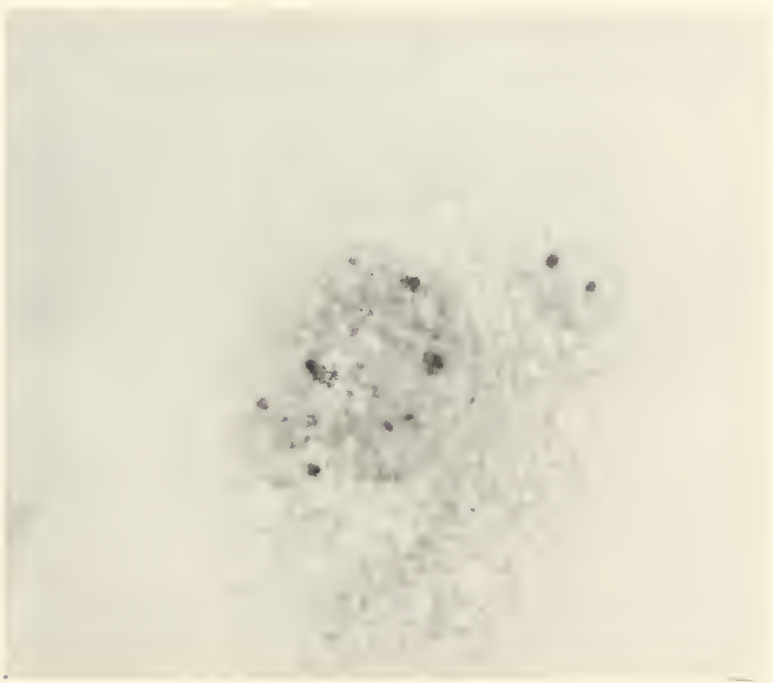


Figure 31. --Scrapie at 151 days. Contrast between size of nuclei in one cell. Hematoxylin-eosin stain. X160.

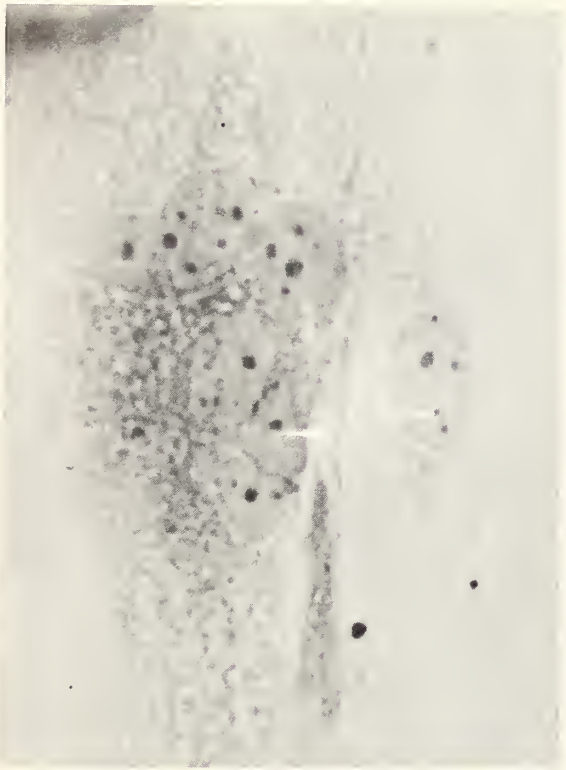


Figure 32. --Scrapie at 151 days. Multinucleation. Hematoxylin and eosin stain. X160.

Figure 33. --Normal at 145 days and 7 serial passages exposed to medium of scrapie culture harvested at 164 days. This culture was fixed and stained with May-Grünwald-Giemsa after 33 days. X160.

Figure 34. --Normal at 98 days and 1 serial passage exposed to medium of scrapie culture harvested at 117 days. This culture was fixed and stained with May-Grünwald-Giemsa after 42 days. X100.

Figure 35. --Normal at 256 days and 4 serial passages then exposed to medium of scrapie culture harvested at 275 days. This culture was fixed and stained with May-Grünwald-Giemsa after 27 days. X160.

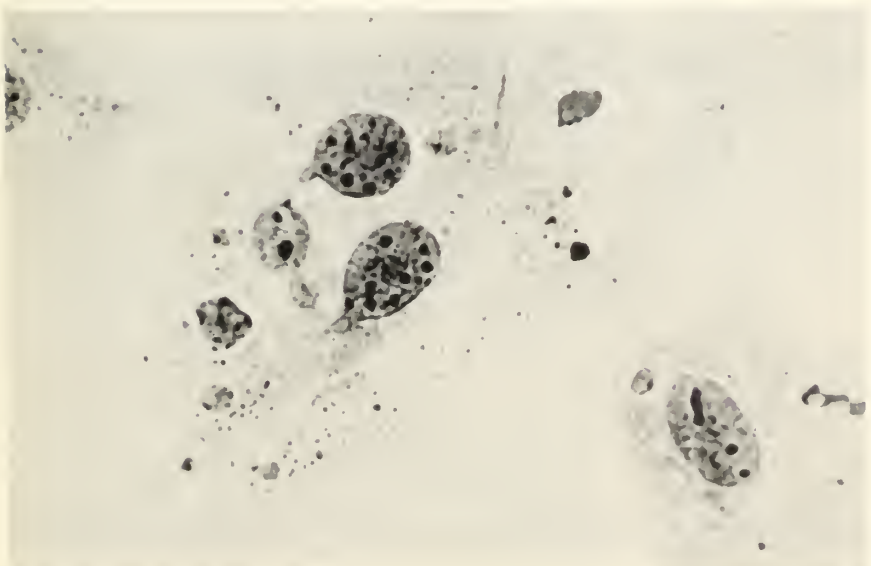


Figure 36. --Normal at 200 days and 3 serial passages exposed to medium of scrapie culture harvested at 219 days. Multinucleation and blebbing. This culture was fixed and stained with May-Grünwald-Giemsa after 29 days. X100.

vested at 35 days from scrapie sheep derived cell cultures developed scrapie. Signs of the disease were convincing and the mice were killed from 508 to 513 days after inoculation. All were males and over the period in which signs of the disease developed, with variations in degree the following signs were observed; waddling gait, lethargy, priapism, soiled perineum, plasticity of the tail, trembling, and somnolence in terminal phase. Histological preparations of the encephalon and the thoracic portion of the spinal cord, stained by Hadlow's modification of Cajal's gold chloride sublimate method, showed astrocytic response characteristic of scrapie in mice. Hypertrophy and hyperplasia of astrocytes was present in many areas of the encephalon such as the thalamus, corpus callosum, dentate gyrus of the hippocampus, and the cranial and caudal colliculi of the mesencephalon. The gray matter of the sections of the spinal cord contained striking numbers of hypertrophic astrocytes especially in the ventral columns.

One of five white Swiss mice (2 males and 3 females) inoculated at 11 days of age with medium harvested at 22 days from a culture of cells derived from the scrapie affected sheep developed scrapie. The mouse, a female, developed signs typical of the disease. The diagnosis based on signs of the disease was confirmed by observation of astrocytosis in histological preparations of brain and spinal cord.

White Swiss and C57BL/6JAX mice inoculated with later harvested and stored medium from scrapie origin cell cultures have not developed scrapie at this writing. Similar mice were inoculated with harvested and stored medium derived from normal sheep brain.

DISCUSSION

The cell cultures from a scrapied sheep have been observed to be different from similar cultures from normal sheep in several ways. The differences involved cellular morphology and colonial characteristics. The differences persisted for more than a year and through 20 or more serial subcultures of the two cell lines. However, the differences diminished during the second year as it became more difficult to maintain and transfer them. Ultimately the culture from the non-scrapied sheep was lost through contamination, however it was declining and may have been lost soon otherwise. At the present the cells from the scrapied sheep, in serial culture for 30 months at the 34th passage, are being maintained with difficulty.

The observation that the characteristics cell morphology and colonial growth could be transferred from the scrapie culture to the non-scrapie origin culture by exposure to cell free medium from the scrapie culture, encouraged the consideration that a transferrable agent was involved in the differences between the two cultures.

Mice developed scrapie when inoculated with media from the scrapie culture harvested 3 and 5 weeks after initiation of the cultures and stored 9 months prior to use. Further experiments, not completed, will help to clarify whether or not the scrapie agent is present in other samples of stored media harvested from time to time in the last 30 months. Samples from the non-scrapie culture are also available for test.

Despite the strong suggestion that the scrapie agent may have been or may still be in the cell culture from the scrapie sheep it remains to be confirmed by further effort.

The observation that the non-scrapie origin cell cultures were altered by transfer of medium from the scrapie culture is of considerable interest and needs confirmation.

Experiments utilizing mouse source material have been initiated and may provide a more manageable system from a source material standpoint.

CONCLUSIONS

Highly suggestive evidence for the presence of the agent of scrapie in subculturable cell cultures from the brain of an affected sheep has been found.

REFERENCES

(1) Bertrand, I., Carre', H., and Lucam, F. (1937) Ann. Anat. path. méd-chir., 14, 565.
(2) Chandler, R. L. (1961) The Lancet, 7191 I, 1378.
(3) Gustafson, D. P., and Stromberg, M. W. (1962) Proc. 66th Ann. Mtg. U.S. Livestock San. Ass'n., 274.
(4) Hadlow, W. J. (1916) Res. Vet. Sci., 2, 289.
(5) Morris, J. A., and Gajdusek, D.C., (1963) Nature, 197, 1084.
(6) Orr, M. F., Hardy, M. H., and Pomerat, C. M. (1950) Anat. Rec., 106, 309 (Abstr.).

DISCUSSION

Dr. Morris:

Are you saying that it is easier to establish a stable line from the scrapie sheep than it is from a normal sheep line?

Dr. Gustafson:

Yes, it seems to be.

Dr. Gibbs:

Do you feel that there is a carrier state that exists with the cell line that you have been carrying with scrapie-infected sheep?

Do you feel that this is a persistent type of infection that is continuing, and can you continue this cell line with the changes that you have noted over a period of time?

Dr. Gustafson:

We are currently trying to determine these by inoculating mice with supernatant fluids harvested from the scrapie cell cultures and from the normal sheep cell cultures.

Dr. Gibbs:

Your original line is continuing?

Dr. Gustafson:

Yes. We established it on February 11, 1962 and when February 11 comes around again in 1964 it will be two years.

Dr. Gordon:

There are no results from your inoculated mice so far with this supernatant fluid--

Dr. Gustafson:

Maybe you can help me when we see the movies.

We have movies of mice that have been inoculated with Sheep No. 374 tissue culture fluids and the normals. I think there is a sequence in the movie which will show the two. We are making histological preparations from the mice inoculated with those fluids and also have another effort in passage in mice. We hope that it will begin to take in mice so that we will be able to confirm or deny--

Dr. Gordon:

We are going to see a clinical syndrome in this picture. Did the mice eventually die or did you have to kill them?

Dr. Gustafson:

They eventually died. We have relatively few mice in the groups on experiment. As a matter of fact, I think we have inoculated a total of 400-some mice in all of our trials.

I think we tried to do a little too much with what we had to start with. We are now interested in getting the strain going in mice before we are able to confirm or deny the possibility of scrapie being in the various harvests from experiments.

Dr. Stamp:

How many scrapie sheep brains have you cultured, and how many normal?

Dr. Gustafson:

One from scrapie; and several normals.

Dr. Stamp:

Do you keep them for this period of time?

Dr. Gustafson:

Yes.

And we are able to do it with pigs as well as mice. It is not a difficult thing for us to achieve. We have more difficulty establishing central nervous system cultures from mice than from sheep. This seems to be related to media, more than to an inherent difficulty.

I think we will show the movies.

(Film presentation)

These are C-57 mice, and are inoculated with The Q-4861 that was received from Dr. Hadlow.

This was in an early phase of scrapie. Of course we find that in the later stages many times we think the mice are actually dead, and they are actually flat in the cages. But when we pick them up they will begin to slowly move.

When we first saw signs of alteration in their activity we began to take some pictures of the mice.

We find the mice have rougher hair coats, altered gait, showing adduction of the pelvic limbs and plasticity of tails, just as has been described by Dr. Morris and shown by Dr. Gordon.

We find that some of them will circle and then circle the other way, and so on.

Some C-57s will sit in the cage with arched backs and fine tremor.

When the mice become aged, it has become more and more difficult for us to determine whether or not they may have scrapie. Some of the controls have considerably altered activity. Some of the white mice have developed tumors which were nearly as large as the mice themselves.

Dr. Dickinson:

None of these are challenged with your own isolation?

Dr. Gustafson:

Oh, yes.

Dr. Dickinson:

Some from Dr. Hadlow and some from--

Dr. Gustafson:

Yes.

Dr. Dickinson:

Could you point out a few?

Dr. Gustafson:

There will be an indication in the card. These are from the 4861, I believe.

Dr. Dickinson:

How long have you been able to observe any--

Dr. Gustafson:

For the ones that I inoculated with your material, possibly, it took four months.

Dr. Dickinson:

How long has it been since these are starting to show anything?

Dr. Gustafson:

I believe this is about four weeks after they began to show signs. When they become more or less inactive, signs have been present for a longer period. We find that the older normal mice also have kinky tails and do not hold them out straight.

These movies were taken probably four weeks after the first sequences were taken.

These males were a little bit rough, they had been in the cage together, and they did a considerable amount of fighting. We used them as controls. They were fairly alert at an age when the inoculates were showing considerably less activity and greater lassitude. These fellows literally chewed each other up.

Dr. Morris:

How many months after inoculation?

Dr. Gustafson:

Thirteen and a half months.

Dr. Morris:

What was the history of the cell culture used to inoculate these mice? How long had the culture been going? How many transfers?

Dr. Gordon:

Are those direct from sheep?

Dr. Gustafson:

This is tissue culture material.

The cell cultures were started in February of 1962, and those were inoculated on November 12, 1962. So they were then nine months and the material that we used was a fresh harvest. We didn't use stored material.

Dr. Stamp:

Was it supernatant?

Dr. Gustafson:

Yes.

Dr. Morris:

Were all the mice inoculated intracerebrally?

Dr. Gustafson:

Yes.

Dr. Gordon:

Have you had histological confirmation?

Dr. Gustafson:

This we are doing. We are somewhat behind you fellows because we started later. As you understand; it takes a bit of time even for a mouse inoculation to provide us with a harvest.

Dr. Dickinson:

I found myself confused at the end there between mice which had gone via tissue culture, using the agent you yourself had isolated, and mice which you had challenged directly without any tissue culture involvement? There were both of these?

Dr. Gustafson:

Yes. We still have concern about whether or not we were actually able to determine that the cell culture fluid itself has induced scrapie in the mice. We are waiting for serial passage to provide confirmation.

Dr. Dickinson:

Could I go back to the others where tissue culture had not come in?

First, I assume these were random-bred mice.

Dr. Gustafson:

Yes.

Dr. Dickinson:

You gave a 13.5 months and then 11 months for a repeat trial. This was the earliest case?

Dr. Gustafson:

Yes.

Dr. Dickinson:

This was roughly what incidence?

Dr. Gustafson:

There were three out of eight in one case, it was relatively low.

Dr. Dickinson:

How long did you observe the negatives, presumed negatives?

Dr. Gustafson:

We kept them all until they actually died.

Dr. Dickinson:

Which would be about how long in your strain?

Dr. Gustafson:

They will live more than a year.

Dr. Dickinson:

Maybe not 18 months?

Dr. Gustafson:

I would need to refer to notes to be sure.

Dr. Dickinson:

In opening these up when you killed them--

Dr. Gustafson:

As I pointed out to you last evening, we went through some of the trials and terrors of the first experiences in raising mice, which involves the ectoparasites you speak of and overheating. Some of them died from those situations. Our results in those situations will cloud the incidence of the disease.

Dr. Dickinson:

I sympathize. What roughly is the pathology here? Is there any intraneuronal vacuolation?

Dr. Gustafson:

As I told Dr. Gordon, the sections are currently being observed, and we have not any sections to present in the way of histological changes.

Dr. Dickinson:

At post mortem, did you notice consistently anything such as an inflated bladder?

Dr. Gustafson:

No.

Dr. Dickinson:

I have one strain that consistently does this. And another that doesn't. I wonder if your strains might relate to this.

Dr. Gustafson:

No, we didn't make note of that at all.

Dr. Wedman:

On your fluid harvest from the normal cells which had been challenged with supernatant, on serial passage do you continue to get a multinucleation or CPE?

Dr. Gustafson:

Yes, this culture will continue with that kind of a presentation.

But, as I told Dr. Morris, as time has passed, we find that the differences between the two cultures is diminishing. We ran an experiment over a 6-week period. There were normal CNS cell cultures from sheep exposed to the Q-4861 mouse brain material, and to cell culture fluid from the sheep scrapie. There were cultures from experimental sheep scrapie and normal sheep CNS cultures for comparison. Each week for 6 weeks, after having initiated these cultures in tubes, slides were fixed and stained for comparison.

The principal differences seen were that there were far more cells in those cultures that were exposed to scrapie source materials and that there were differences in cellular morphology. I wouldn't want to try to prove it in any court, at this point.

These observations await confirmation on the basis of inoculation of mice, supplemented by histological changes in mice.

The cellular differences are not always as pronounced as those shown in the slides of the normal and the scrapie sheep, or between materials from a normal mouse and scrapie mouse.

Dr. Wedman:

When you harvest fluid from the normal, which has produced the multinucleated cells, then going back into another not serial transfer but another set of normal cells--

Dr. Gustafson:

I have not done that.

Dr. Wedman:

My question is going to be, have you been able to see a shortening period of CPE?

Dr. Gustafson:

No, because we have not done this.

SCRAPIE, NATURAL AND ARTIFICIAL: THE NATURE OF THE PROBLEM

H. B. Parry[1]

In 1952 the published accounts of scrapie in Great Britain provided a very inadequate account of the actual manifestations of the disease and very little detailed information about its occurrence was available, since sheepmen were extremely reluctant to admit to any first hand knowledge of the disease. Such verbal accounts as were available indicated that the disease showed great variations in incidence in different flocks and breeds, and that the clinical manifestation of the disease varied more than was described in the literature.

The most pressing requirement was the assembly of accurate and comprehensive data within a group of flocks, preferably a closed or semi-closed breeding population, of all forms of chronic neurological and neuromuscular illness over a period of years, to permit a more comprehensive and accurate description of the natural disease. This necessitated the introduction of special methods of recording and the establishment by voluntary arrangement of closed breeding populations or breeder groups.

From these data it became apparent that the clinical form of scrapie could manifest itself in several different syndromes, in a number of which a myopathy of the skeletal muscles of polymyositis type was present (2). However there were a number of clinical features, for which no proper pathology had then been found, although much work had been done on the distribution of vacuolated neurones (6). A more thorough examination of the nervous system, which had not been completed in 1956, revealed a specific primary system degeneration in two specialised neuro-anatomical systems, the olivo-ponto-cerebellar system and the hypothalamo-neurohypophysical system (1). These findings provide a probable pathological basis for many of the motor signs and disturbances of the homeostatic mechanisms of the body seen in scrapie, but there remain others, such as the rubbing and blindness for which there is at present no satisfactory explanation. This wider concept of the pathological and clinical bases of scrapie brought the disease into the sphere of a number of chronic neuromuscular disorders of man. At the same time the epidemiological data pointed to the importance of a genetic predisposition in the aetiology of the disease. The field data available (4) were consistent with the view that the clinical manifestation of scrapie was controlled by an autosomal recessive gene. Yet in natural cases of scrapie of this presumptive genetic type, the transmissible agent first reported by Cuillé & Chelle (3) was present.

In any proper consideration of scrapie, it is essential to consider the problem in its entirety and to attempt some synthesis of all the laboratory findings with the direct observations of the natural disease in the field. A working hypothesis based on such a synthesis of the knowledge then available was put forward (5) in which it was suggested that in the causation of natural scrapie in sheep there were two components, a lethal recessive gene manifesting in middle age, and an infective but not infectious agent closely associated with the gene, which could be transmitted artificially but which did not appear to spread under natural conditions in the field, i.e. scrapie was not an infectious disease of the common communicable type.

In discussing the various aspects of scrapie, it is helpful to distinguish clearly between the natural disease, which may be spontaneous or be produced experimentally by suitable

[1]Nuffield Institute for Medical Research, University of Oxford, Oxford, England.

matings, and the artificial post-inoculation disease, i.e. the disease set up originally by artificial inoculation of experimental animals, and its subsequent occurrences.

When speaking of the natural disease and of the recessive gene hypothesis, it is convenient to classify sheep as shown in figure 1.

The word infective is used to denote a situation in which a transmissible agent has been demonstrated but for which the evidence for spread by contact, mediate and vector contagion, coitus or residual contamination of premises is equivocal, and its importance in field dissemination remains in doubt. The term infectious is reserved for a situation in which such evidence is quite unequivocal and the usual method of dissemination is by contact, etc.

The studies reported in this and subsequent papers have been made possible by generous grants to the University of Oxford from the Nuffield Foundation of London and the National Foundation for Neuromuscular Diseases of New York.

Scrapie status – individual assessment.

Affected – developing scrapie at any age.

Unaffected – under observation to 4½ yrs.

Unclassified – not under observation to 4½ yrs.

Black – affected or both parents affected.

Grey – one parent affected or with affected progeny.

White – (a) breeds without any evidence of scrapie (eg SC1 and 2).
(b) in breeds with scrapie.

off-white – (i) unaffected with neither parent grey or black or close relatives affected.

chinese white. { (ii) test-mated clear of the scrapie gene.
(iii) the progeny of two test-mated clear parents.

Figure 1.

REFERENCES

(1) Beck, E., Daniel, P. M. & Parry, H. B. 1962. Hypothalamic and cerebellar system degeneration in sheep with 'scrapie'. Proc. IV Int. Congr. Neuropath., München, 3, 269-274.

(2) Bosanquet, F. D., Daniel, P. M. & Parry, H. B. 1956. Myopathy in sheep. Its relationship to scrapie and to dermatomyositis and muscular dystrophy. Lancet, ii, 737-746.

(3) Cuillé, J., and Chelle, P. -L. 1936. La maladie dite 'tremblante du mouton' est-elle inoculable? C. R. Acad. Sci., Paris, 203, 1552-1554.

(4) Parry, H. B. 1960. Scrapie: a transmissible hereditary disease of sheep. Nature, Lond., 185, 441-443.

(5) Parry, H. B. 1962. Scrapie: a transmissible and hereditary disease of sheep. Heredity, 17, 75-105.

(6) Zlotnik, I. 1958. The histopathology of the brain stem of sheep affected with natural scrapie. J. Comp. Path., 68, 148-166.

Scrapie status – individual assessment

Affected – developing scrapie at any age.

Unaffected – under observation to 4½ yrs.

Unclassified – not under observation to 4½ yrs.

Black – affected or both parents affected.

Grey – one parent affected or with affected progeny.

White – (a) breeds without any evidence of scrapie (eg SC1 and 2).
(b) in breeds with scrapie.

off-white – (i) unaffected with neither parent grey or black or close relatives affected.

chinese white. { (ii) test-mated clear of the scrapie gene.
(iii) the progeny of two test-mated clear parents.

NATURAL (SPONTANEOUS) SCRAPIE IN SHEEP I. CLINICAL MANIFESTATION AND GENERAL INCIDENCE, TREATMENT, AND RELATED SYNDROMES

H. B. Parry[1]

INTRODUCTION

Scrapie is a chronic, wasting disease of sheep known in Britain and Western Europe since 1780 (Comber, 1780)[2] and probably since 1720 (see M'Gowan, 1914).[2] It is always fatal and has from time to time been responsible for the elimination of the sheep population of a district. It has recently been recognised in North America and the Antipodes.

The cause of scrapie has been for long a matter of dispute. The incidence of the disease in Western Europe has shown striking fluctuations in a region over a period of years (see M'Gowan, 1914). In the 18th and early 19th centuries many writers considered scrapie to be hereditary, but during the past half century the view that it was a naturally infectious disease has gained ground, supported since 1936 by studies of experimental transmission by inoculation.

Precise knowledge of the natural disease has been difficult to assemble. The sheep-owners are reticient, clinical manifestations vary considerably, no simple laboratory tests are known and the pathologic lesions, often inconspicuous, have been considered non-specific. However, recent work (Beck, Daniel and Parry, 1961) has shown that there are specific degenerative changes in the brain in the hypothalamic-hypophyseal system and in the olivo-ponto-cerebellar system.

Data have been collected relating to 1,400 cases of which over 1,200 have detailed individual records. They provide evidence of 3 kinds; first, that the prime cause of scrapie is heredity and that only individuals of the homozygous recessive geno type manifest the disease; second, that affected animals harbour a substance, the transmissible scrapie agent, which is infectious artificially, and third, that scrapie is not naturally infectious (Parry, 1960).

CRITERIA OF THE DISEASE

Clinical Signs

The various clinical forms of scrapie have been discussed by Bosanquet, Daniel and Parry (1956) and Parry (1957); only cases of their Group A syndromes, which comprise more than 95% of all cases, have been accepted.

In these syndromes rubbing of the buttocks, flanks and head is conspicuous and may be almost continuous in some advanced cases; in others nibbling of the haired skin of the lower legs is marked, while nearly all animals exhibit a pronounced nibbling reflex on deep palpation of the soft tissues of the back. In many an acneform papular rash appears on the haired portions of the skin, notably of the lower legs and nose, and haematomata of the ears are common.

Nearly all cases show early fatigue with moderate exercise and in some this asthenia progresses rapidly to general weakness with inability to rise. Progressive loss of bodily condition leading to emaciation, in spite of a food intake adequate to maintain body weight, is conspicuous in the majority of cases; a few increase in weight and die obese. Excessive thirst is not infrequent.

Many show disturbances of gait, notably of the hindlimbs, which are wide-based, and a high

[1] Nuffield Institute for Medical Research, University of Oxford, Oxford, England.

[2] See reference, page 103.

stepping movement of the forelimbs may occur ('Trauberkrankeiten' of German writers); if the ataxia is severe, the animals reel like a drunken human. In some cases there is in addition a disturbance of postural fixation leading to a coarse tremor or trembling ('La Tremblante' of French writers), which is accentuated by excitement. The body righting reflexes are unaffected until an advanced stage. Tendon reflexes are normal or even exaggerated, and the normal response to pin-prick is very rarely absent.

Mental disorder leading to personality changes and even dementia occurs frequently. Many become uncooperative and refuse to be driven some become fearless of humans while others become very excitable and are extremely distressed by any handling or change in their daily routine. Not infrequently a minor behavioural abnormality may be the first sign noted. Blindness occurs in some 5 percent of cases.

Body temperature and heart rate show unusual fluctuations although within normal limits, but the cardiovascular system is generally unstable. Advanced cases are particularly susceptible to cold and wet. The 5 principal and typical syndromes are summarised in Fig. 1; they account for at least 95 percent of our cases.

The disease is almost always slowly progressive with death in 6 weeks to 6 months, rarely a year. Very occasionally complete remission of signs occurs. 3 such cases, 2 rams and a ewe, have been seen, but they have all died of scrapie, the longest intermission being 3 1/2 years from the age of 2 3/4.

In populations with a high presumptive scrapie gene frequency, unusual syndromes of palsy, wasting and myasthenia of uncertain aetiology occur alongside cases generally accepted as scrapie, with which they appear to be closely associated. These are summarised in Fig. 2 and may account for up to 5 percent of all cases of scrapie-like disease.

At autopsy few macroscopic abnormalities are present. Conspicuous myopathic lesions seen in the early cases of the series have been absent in many recent cases, although a high proportion of animals show abnormalities of the skeletal musculature. The adrenal glands are usually enlarged and the thyroid glands small. Chromatolysis and vacuolation occur in the neurones of the central nervous system.

Typical syndromes

1. Classical scrapie.	Rubbing. Ataxia Wasting of skeletal muscles. Dementia variable.
2. Non-wasting scrapie.	Ataxia and asthenia. Rubbing. Obesity. Dementia.
3. Pining scrapie.	Wasting marked. Rubbing. No ataxia. Absence of mental signs.
4. Ataxic scrapie.	Severe ataxia. Wasting, Rubbing } very slight. Some dementia.
5. Amaurotic scrapie.	Visual defects. Ataxia. Wasting, Rubbing } often absent. Dementia.

Figure 1.--A summary of the principle clinical signs in the five typical syndromes of classical natural scrapie in sheep.

Atypical syndromes

6. Palsy - hindlimb.	Loss of power in hind-limb leading to inability to rise No rubbing, wasting or mental changes
7. Palsy - forelimb.	Acute loss of motor function of forelimbs Some mental changes Rubbing as terminal sign
8. Acute general asthenia.	Severe general muscle weakness No rubbing or wasting.
9. Idiopathic pining.	Wasting and wool changes. No rubbing or ataxia
10. Miscellaneous	Epileptic seizures Dementia. Defect of eye movements.

Figure 2.--A summary of the principal clinical signs in five atypical syndromes of natural scrapie-like illness in sheep.

Diagnosis

The diagnosis has been based on the following criteria, (1) unequivocal clinical signs; (2) a fatal outcome in all animals retained under observation; (3) lesions in the skeletal muscles and changes in the endocrine glands; (4) cytoplasmic vacuolation and neuronal degeneration

in the nervous system; (5) the demonstration of a transmissible agent in the brain. (6) the occurrence in close relatives and progeny as if due to a recessive gene.

It has not been practicable to apply all these criteria except in a few cases. Of the 1,400 animals affected, 650 have been seen by the author, while in the remainder, the diagnosis has been based on the clinical observation of others with experience of the disease. Some errors of diagnosis will have occurred, but their number is probably small, since all cases have been discussed with the observer within 1-2 months, occasionally up to 6 months, of the illness occurring, and any case in which the evidence was unconvincing has been excluded. These cases have been used only in assessment of general incidence. In all special studies for statistical and genetic analysis, the first cases in any flock and doubtful cases have been examined personally and confirmed neuropathologically. All the cases examined fully have conformed to the above criteria; only a very small number have been assayed individually for the presence of the transmissible agent. Figure 3 summarises the main points.

SOURCE OF MATERIAL

The principal data have been collected over the years 1952 - 64 from a group of some 50 recorded pedigree sheep flocks of 10 breeds, with over 10,000 breeding females in 1964; more than 20,000 animals have been under observation during the period. The flocks are dispersed in 17 counties of England and Scotland on varied soil types; they have a very high standard of shepherding, and are members of a Stud Sheep Health and Improvement Scheme, known as the Oxford Scheme (Parry, 1957); now the Sheep Development Association and British Sheep Society (1963).

Additional data have been obtained from other commercial flocks in which individual animals are not recorded. The Oxford Scheme flocks are visited at least twice a year, and their records are supervised closely. In these flocks individual animals are identified by one and usually two independent methods of permanent identification in addition to temporary fleece numbers and marks; records are kept of matings, births, markings and all cases of ill-health, death and disposal. These records cover all the stud rams and breeding females, but

Diagnosis of field cases

Class of flock	Nature of data being assembled	Clinical diagnosis: Experienced stockman	Clinical diagnosis: Veterinary neurologist	Pathological confirmation: Specialist neuropathologist
A. In supervised flocks				
1. affected	General incidence	Yes	Periodical confirmation	No
	Special studies	Yes	Yes	Yes (blind)
2. unaffected	First cases especially rams	Yes	Yes	Yes (blind)
B. In other flocks	Ancillary evidence	Reliability variable	Essential	Desirable

Figure 3.--A summary of the diagnostic criteria used for general and special of natural scrapie of sheep.

only a small proportion of their progeny, namely the females retained in the flock; these represent usually 20 percent to 25 percent of the females born, and are selected when 1-1/2 years old, partly on inspection for the main breed points and partly on the performance records of their relatives. The males and surplus young females are sold before they are 2 years old and only occasionally is it possible to follow their subsequent history. Older females may be removed from the flock at any time if they fail to perform satisfactorily or suffer some acquired disablement, although good breeding animals are kept until they have had 5 or more gestations and are 7 to 10 years old.

These flocks normally breed all their own females but purchase stud rams from other flocks in the Oxford Scheme. The rams are selected mostly when 6 to 8 months old for good growth and muscular development with satisfactory wool and breed conformation. They are first used as sires when about 8 months old and kept for 2 or 3 years, occasionally until 6 or 7 years old. As these flocks comprise the principal ram-producing flocks of their breeds, the stud rams tend to have a number of common ancestors, which have been outstandingly successful sires in their breeds; thus many rams will have at least one great grandparent in common. There is therefore a tendency to a concentration of certain blood lines in each breed, and for a high degree of consanguinity to be prevalent. Each flock-group is virtually a closed breeding population, a matrilinear breeding group, with a relatively high index of inbreeding and extreme polygamy.

These flocks are free from the common infectious diseases, except contagious pustular dermatitis. Stillbirths and other neonatal mortalities are unexceptional and severe congenital anomalies rare, being less than 5 per 10,000 lambs born. There have been no reasons to suspect foetal death early in gestation other than on odd occasions. The general health record of these flocks is high; the annual loss of breeding ewes from all causes other than scrapie is less than 5 percent; the percentage of ewes pregnant more than 95 and only sporadic abortions not associated with infections have been observed. The average litter size is 1.6 to 1.8 and the reared lamb ratio 1.4 to 1.5 lambs per ewe put to service.

For many years some flockmasters have endeavoured to dispose of (i.e. cull) any near relatives of sheep showing signs of scrapie or suspected of being affected. Since 1952 the majority of full and half brothers and sisters, daughters, sons, mothers and fathers of affected animals have been culled but no attempt has been made to quarantine affected sheep; they have remained in the main flock until it was convenient to dispose of them.

This general information relating to groups of the principal flocks of the breeds has been supplemented by a more detailed special studies of several flocks with good records, especially Flock A, for which exceptional records are available for over 20 years. This flock has been self-contained for females since 1940. There has been one shepherd who has kept a notebook for each lambing season in which the tupping groups and other details were noted. As each ewe lambed, the date, her health, milk flow and particulars of parturition were noted, together with the number, sex and condition of the lambs, their sire, and a diagram of the ear marks inserted. These notebooks are available from 1939. From 1940 onwards a standard method of marking all sheep by a system of numbering by ear-notching carried out in the lambing coops within 2-3 days of birth, followed before they were 18 months old by tattooing a year letter with the ear notch number into one ear and the breed emblem and registered number of the flock into the other ear. Latterly each breeding ewe has had a fleece number branded on her side, so that she may be readily identified without the necessity of examining the ear marks closely. From these notebooks it has been possible to compile a comprehensive record of the flock with exceptionally few discrepancies, for when a mating has been in doubt this has been recorded. The shepherd had kept a list of all cases of scrapie since the first one was noted in 1945, while since 1952 nearly every case had been examined clinically by the author, and has been kept in hospital under observation, and latterly, all have been examined at autopsy. Similar records are available for Flock B, but not all the early cases of scrapie were recorded.

As public knowledge of the occurrence of scrapie in a flock may have serious financial and social consequences for both flockmaster and shepherd, the data to be presented are

given in a form which is designed to obscure their precise origin.

INCIDENCE

Terminology

The birth-group comprises all animals born into a flock in one breeding season, usually of 12 months; in sheep breeding twice a year the period is 6 months.

The age-group includes all animals of a given age, normally covering one year, and commencing when an animal is 6 months of age e.g. an animal between 6 and 18 months of age is in the 1-year old age-group.

The attack-rate is the number of manifest cases of scrapie per 100 breeding ewes occurring in a breeding year. The incidence, expressed as a similar percentage, is a less precise term used to describe the approximate manifestation over a period of years.

A flock or flock-group is considered to be free if over 5 years or more no manifest case has been observed in the home sheep and no case in sold sheep has come to notice. If affected, a sporadic incidence is 1 percent, a low incidence 1 - 3 percent, a medium incidence 4 - 10 percent, a high incidence 10 percent and above.

General

4 flock-groups, 3 pure-bred and 1 cross-bred have adequate and supervised records for 5 years or longer; one of these groups is free of scrapie; 2 of the affected flock-groups have a low to medium incidence, while in the third it is sporadic. 5 other flock-groups with good records, but not yet supervised for 5 years, are probably free. Thus the incidence varies very much between different flock-groups, although direct and indirect contact between animals from affected and unaffected flock-groups occurs frequently at Agricultural Shows and on certain farms. It occurs in pure-bred and cross-bred sheep, of long and short-wooled breeds, on mountain and lowland.

By Flocks and Flock-groups

Of 12 free flocks, 9 have used many rams from flocks with affected animals during the period of the rams' breeding and rearing, while in 2 flocks stud rams have manifested scrapie at or shortly after the mating period without any further case developing in 3 and 5 years respectively. In 9 flocks an occasional case has occurred giving a sporadic incidence, often with periods of several years without a case.

Among the initial high incidence flocks the attack-rate has declined in 3 from 8.6 percent to 1.8 percent, while in 2, in which several stud rams used extensively were subsequently affected, it has remained about 10 percent. In one flock thought to be free an attack rate of 15 percent appeared suddenly in females of certain families, but suspected cases in sold sheep had been reported for several years.

The striking feature is the wide fluctuations in the attack-rate in different flocks over a period of years and even from year to year, the disease disappearing and reappearing capriciously, the flock meanwhile being exposed to apparently similar environmental factors. Many flocks remain free in spite of using rams from affected flocks. In some an occasional sporadic case occurs, but the disease does not spread; in others with no recorded cases for years the disease may appear suddenly as an epidemic.

By Age of Individuals

Adequate records are available for 1008 affected animals, 930 ewes and 78 rams. The small number of rams recorded is due to the small sample under observation; only one ram is retained for every 20-40 females and until recently the majority of stud rams have been disposed of before 3 years of age.

The age of onset of unequivocal signs were classified by 1/2 yearly intervals. The results are shown in Figs. 4 & 5 which demonstrate that the great majority of cases manifest between 2 and 7 years of age.

The data for rams show that they tend to show manifest signs at a rather earlier age than ewes and fewer cases have been observed in old rams.

Figure 4.--The age, in six-month periods, at which unequivocal clinical signs of scrapie were first manifest in 1008 sheep.

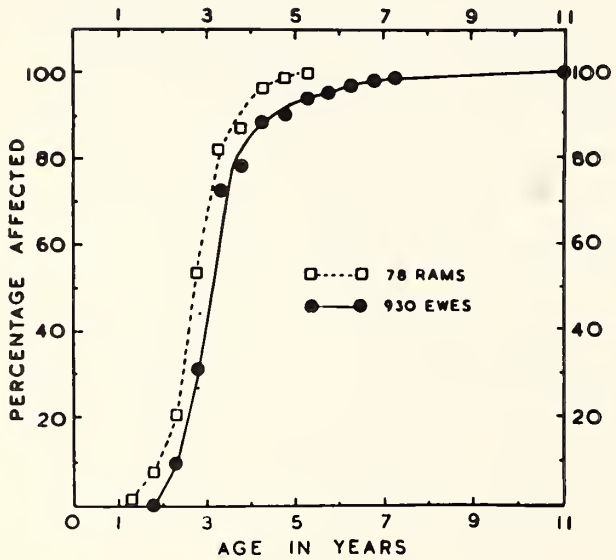


Figure 5.--Curves showing the cumulative manifestation for rams and ewes based on the percentage of all animals of each sex affected by a given age.

Table 1 shows the age distributions obtained for the first series of 526 cases (sample B) collected up to June 1958 (Parry, 1960), with that for the second series of 482 cases (sample C) collected between July 1958 and December 1960, and for the total sample of 1008 cases (sample A). Sample D are the progeny of two affected parents. It will be seen that the age group distributions are remarkably close, indicating a constant rate of onset and a high repeatability of the observations. The age distributions for 2 different flock-groups each with over 100 cases are very similar.

The close fit to the distribution age of the data for females in sample D with the general suggests that the genotype of the parents is not important in determining the age of manifestation in any ss offspring, and this is confirmed by data for 52 affected animals in flocks A and B. 13 had both parents affected, 15 had one parent affected and one a heterozygote and 26 had 2 heterozygous parents. The median age of onset for each group was 3-1/4 years, with mean ages of 3.4, 4.0, and 3.5 years, respectively.

Many sheepmen maintain that females first pregnant at 8 months old instead of the more usual 20 months develop the disease earlier. We have been unable to confirm this in two flocks in an age-group at present 3 years old.

Table 1.--The age of manifestation, expressed as the percentage of those affected, in the total sample A, in the preliminary sample B (1945-58), and in the current sample C (1958-60). Sample D contains those individuals from samples B and C which were the progeny of two affected parents, and all of whom are expected to manifest (see table 3)

Sample	No. of animals	Percentage of the sample manifesting at this age in years								
		1/2-1 1/2	1 1/2-2 1/2	2 1/2-3 1/2	3 1/2-4 1/2	Total to 4 1/2	4 1/2-5 1/2	5 1/2-6 1/2	6 1/2-7 1/2	Over 7 1/2
Rams										
A	78	1.3	19.2	61.6	14.1	96.2	3.8	0	0	0
B	34	2.9	20.6	58.9	14.7	97.1	2.9	0	0	0
C	44	0	18.2	63.6	13.6	95.4	4.6	0	0	0
D	7	0	42.9	42.9	0	85.8	14.2	0	0	0
Ewes										
A	930	0	9.4	63.5	15.6	88.5	5.4	3.1	1.7	1.3
B	492	0	7.3	63.8	16.3	87.4	5.5	4.1	1.8	1.2
C	438	0	11.6	63.2	14.9	89.7	5.3	2.0	1.6	1.4
D	45	0	8.9	71.1	13.3	93.3	4.5	0	2.2	0

In one flock, of 10 affected animals 5 lambed as lambs and 5 did not; in the second of 12 affected, 7 lambed as lambs and 5 did not. The youngest cases in both flocks occurred in animals which did not lamb as lambs; 7 of the 22 cases were manifest before 2 1/2 years of age but only 2 had lambed as lambs.

The age of manifestation does not appear to decrease in successive generations, as the incidence of the disease increases, i.e. 'anticipation' does not occur. Thus in flock A, the median age of animals affected in the flock year was 4 1/4 years when the disease was first noted in 1945, was 3 3/4 years in 1950 and 3 1/4 years in 1951, since then the median age has remained at 3 1/4 years except in 1956 when it was 2 3/4 years. Records of other flocks are in general agreement with this conclusion. However, in 6 cases a daughter or son has died of scrapie 12 months or more before their sire or dam was affected and in one instance in flock A 3 daughters were affected 2 months before the sire was affected. Such examples may be attributed to the normal variation in age of onset and the short intergeneration interval, often of only 1 year on the male side, rather than to specific 'anticipation'.

By Sex of Individuals

Although the data for rams are less comprehensive than for ewes, there is no clear indication that they are less prone to manifest the disease than the females. Where the male progeny of affected parents have been under observation, they have shown an attack-rate very similar to that of the females. The distribution of the age of manifestation is also very similar (see Fig. 4).

By Season of the Year

Figure 4 shows that more cases occur in the first 6 months of the calendar year, the majority manifesting between October and March, i.e. midwinter in Britain. This coincides with late pregnancy and lactation in the ewe but follows the period of reproductive activity in the ram. It seems likely therefore that some common environmental factor other than reproductive activity may play a major role. This could be nutritional stress, for starving sheep suspected of the disease is employed by some shepherds as a means of hastening the appearance of clinical signs.

THE 90 PERCENT MANIFESTATION LEVEL

Scrapie appears principally in sheep aged 2 1/2 - 3 1/2 years old (Figs. 4 and 5, table 1) and less frequently in older animals. However, cases do develop in older sheep, an important point which will not come to light unless older animals are retained in the flock. For practical purposes it is not feasible to keep large numbers of these older sheep under observation to determine the complete manifestation. However, 89.4 percent of our sample of affected animals were manifest by 4 1/2 years of age. If this figure represents a true estimate of the age distribution of manifestation, then we may accept observation to 4 1/2 years old as revealing 90 percent of all potential cases, while failing to disclose 10 percent, the cases of late manifestation.

The validity of this assumption can be criticised on two grounds. Firstly, if in some flocks or flock-groups the age distribution departed substantially from the normal. From our data this is not so. Secondly, if very few animals older than 4 1/2 years had been under observation in the flocks providing the samples quoted in Table 1 and illustrations the true incidence of late manifestation could be seriously underestimated.

In the great majority of these flocks, however, 15 percent to 20 percent of the breeding ewes are older than 4 1/2 years. Further, it is only in a small number of flocks in which losses from scrapie are unusually high that there are fewer than 15 percent of old ewes. Fig. 6 shows that in flocks A and B the number of ewes over 4 1/2 years old has exceeded 25 percent of the ewes at risk, while having fewer cases than the smaller age-groups of 3 and 4 year olds.

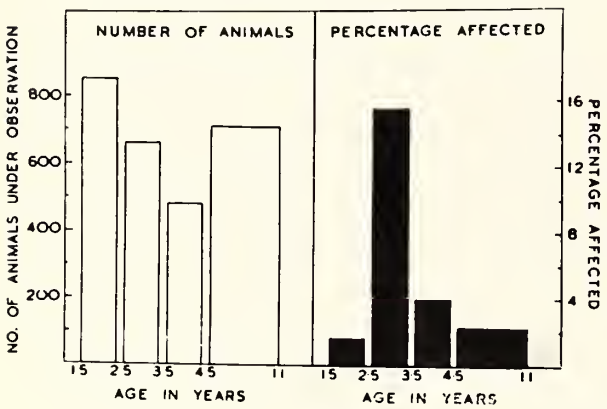


Figure 6.--The number of ewes of four age-groups kept in flocks A and B during the years 1940-56 with the percentages of each age-group manifesting scrapie.

For males so few are under observation over 4 1/2 years of age that it is difficult to assess the importance of late manifestations, although they manifest earlier than females (Fig. 5). However, our data are concerned so largely with females that any error due to under-estimating late manifestation in males will not seriously distort our age distribution.

We have therefore taken 4 1/2 years of age as the 90 percent manifestation level for all potential cases of scrapie, and have used this age as an arbitrary end-point at which to assess all sheep under observation.

An animal alive and well at 4 1/2 years old and thereafter as long as it may be under observation is termed unaffected. An unaffected animal not under observation until 4 1/2 years of age is called unclassified. An affected animal is one showing unequivocal clinical signs at any age.

TREATMENT

No treatment is known which will arrest and reverse the disease, once frank clinical signs have appeared. It is possible that the progress of the disease may be retarded by careful nursing and good feeding if animals are housed in individual pens in a warm, draught-free building.

We have tried, in addition to good nursing and feeding, each of the following treatments on one or two animals, but without any sign of modifying the expected course of the disease:-

Vitamin B_1 given subcutaneously as 1 g. per day of aneurine hydrochloride in aqueous solution for two months.

Vitamins B_1, B_2, B_6, and nicotinamide given as a combined preparation intramuscularly which provided 20 mg., 8mg., 8mg., and 80mg. respectively of the four vitamins twice daily for six days.

A supplement of high-quality protein, minerals, and accessory food factors fed in the form of dried liver and spray-dried whole milk powder at levels of 37 to 75 g. and 200 to 300 g., respectively, for 40 to 50 days.

Vitamin E as α-tocopheryl acetate given subcutaneously as 350 mg. in arachis oil daily for 32 days and thereafter 200 mg. daily per os for 20 days.

Vitamin B_{12} given subcutaneously in doses of 2 mg. daily for four days and thereafter 250 μg. daily for 15 days.

Desoxycorticosterone acetate given subcutaneously as 15 mg. in oily solution daily for 35 days.

Progesterone given intramuscularly as 20 mg. in oily solution daily for 50 days.

Adrenocorticotropic hormone given subcutaneously in a dose of 40 I.U. twice weekly for three weeks.

Three 5-day courses of British anti-lewisite (B.A.L.) with an interval of two weeks between courses.

Neostigmine in single doses of 2.5 to 5.0 mg. intramuscularly may sometimes provide a slight, very transient improvement in the early stages.

The results of these pilot therapeutic trials suggest that the disease is not a peripheral neuropathy due either to a deficiency of the vitamines of the B-complex or to a chronic intoxication with heavy metals such as arsenic, which might be expected to be relieved by B.A.L.; that it is not a muscular dystrophy of vitamin E controlled type nor a general dietary deficiency disease; that it is not an endocrine disturbance readily amenable to the replacement therapy given, nor a primary neostigmine-alleviated disorder of the myasthenia gravis type.

Reference

Parry, H. B. 1962. Scrapie: A Transmissible and Hereditary Disease of Sheep. Heredity, 17, 75-105.

Discussion

Dr. Goode:

Are there any questions or comments regarding the previous two papers?

Dr. Dickinson:

Dr. Gordon referred yesterday to certain situations which I'd like you to classify. I will be referring to more of this type. That is, when you challenge the dam and don't challenge that ewe's off-spring and that lamb goes down with scrapie, where do you put it in this scheme you have on the blackboard?

Mr. Parry:

I would put this within the artificial category.

Dr. Dickinson:

And why?

Mr. Parry:

Merely because you are dealing with the post-inoculation disease. I presume you are. Unless you know what the genotypes of your foundation stock are.

And I'm faced with this one myself. I'll talk about this. I have some animals I don't know where to place, and hence I do not know how to interpret my data.

Dr. Stamp:

Could I ask a question and make one or two comments to the question, if I may, Mr. Parry?

First, the question is what criteria do you, in fact, use for the diagnosis of scrapie? That is the question.

And the comments around this: You made great play, for instance, on the fact that the earlier records were inadequate and that since your work they have been better. I would suggest that in your early publications you refuted the idea that the brain pathology was, in fact, diagnostic of scrapie, and yet today on numerous occasions you have instanced your neuropathologists doing the diagnosis.

In your earlier papers you used the muscle lesions which were very obvious and very clear. Even other than a pathologist, could have identified them. And yet do you now use these obvious lesions as a diagnosis for scrapie? If so, then we don't need a neuropathologist. All you need is a pathologist.

On the clinical side, I think it's well accepted in both veterinary and in medical practice that the diagnosis of disease by clinical methods, and especially neurological disease, in fact leads to a considerable amount of confusion.

Willis, in his recent book on pathology makes the point very clearly that anyone who tries to define a neurological disease without a neuropathological basis is on very, very dangerous ground.

I would like to ask a second question.

On your numerous clinical classifications for scrapie, and they are complex, you yourself say it requires an expert to carry out the diagnosis. No shepherd could possibly do it. Then on what basis do you classify these as scrapie?

Is it on muscle lesions? On brain lesions? Or merely on the fact that these are unclassified diseases found on your scrapie farms?

Mr. Parry:

We shall answer those questions in a moment. They are inherent in the next paper.

Dr. Stamp:

May I have one or two brief answers at the moment before we get confused.

You have put these facets over as being part of the scrapie syndrome? I want to know on what basis you classify these as scrapie.

Mr. Parry:

On their clinical, pathological, genetical and epidemiological basis.

Dr. Stamp:

If this is so, Mr. Chairman, may we know what these--

Mr. Parry:

You will know. I am going to tell you later this morning.

Dr. Stamp:

But you have already confused the picture. I want to know why you classify these as scrapie. Briefly tell me whether it's by neuropathology or tell me whether it's clinical--

Mr. Parry:

I have merely described to you, Dr. Stamp, the clinical syndromes. I intend now to come on and tell you what the neuropathology is as we see it. I wanted to establish what the clinical signs were in these syndromes which we would have to try and explain pathologically.

Dr. Stamp:

Mr. Chairman, if I may say so, I would object to the diagnosis if it was in fact made purely on clinical signs.

Dr. Goode:

I would like to ask one question at this point then. Mr. Parry, as we move on do you propose to step further beyond the clinical diagnosis and get into the aspects Dr. Stamp has just referred to?

Mr. Parry:

Certainly, Mr. Chairman. The next paper deals with the neuropathology. I have broken it up in this way deliberately so that each stage could be discussed. And if anyone wasn't clear about the clinical signs or the basis on which I classify animals as affected or unaffected, it might be made quite clear.

If I try to explain all the features of diagnosis that we adopt at this stage, it's nigh impossible.

You will notice that I have a paper in which I am going to define quite clearly and categorically how we diagnose scrapie or what we think the criteria should be for the diagnosis of scrapie.

Dr. Gordon:

Mr. Chairman, will you be exposing the same slide, Mr. Parry, with the list of clinical signs that you have recorded there and tell us at the time that you do this next presentation what your final diagnosis was based on other than the clinical signs on the slide that you have depicted?

Mr. Parry:

Certainly, Dr. Gordon. You shall have any slide you like.

Dr. Gordon:

This then will be quite helpful, because you give a list of different clinical syndromes, and it will be interesting to have a pathology to associate with these syndromes?

Mr. Parry:

You will indeed.

Dr. Stamp:

I'm sorry, sir. I must press this point. We are going to listen for a whole day today while Mr. Parry talks about scrapie. I suggest that it would be a very good idea, instead of waiting until Thursday morning, that Mr. Parry define this morning what he means by scrapie. We cannot listen for a whole day to what Mr. Parry thinks is scrapie unless we know what he's talking about. I want to know this morning, in front of this audience, what Mr. Parry calls scrapie.

Mr. Parry:

This is perfectly in order with me, Mr. Chairman, if you don't mind me switching material around.

Dr. Goode:

You mean it would be perfectly in order for you to proceed to outline briefly this material that Dr. Stamp has requested? I think this would be quite satisfactory. I think the question is quite clear and certainly an objective one. It would be helpful if it doesn't interfere appreciably with your presentation.

Mr. Parry:

All right. It is going to come out almost immediately, yes.

Mr. Yohe:

On some of your atypical cases here on syndromes you mentioned such things as wasting. Do these sheep proceed to death along this line and never show any other symptoms but wasting, or in some cases merely incoordination of muscles, and so on?

Mr. Parry:

They will proceed to gradually waste away and die without any ataxia, without any rubbing, without any focal neurological signs that I have seen, and without, as far as I can detect, any reason for the wasting. That is, their food intake on the whole is more than adequate to maintain their body weight on the usual accepted standards. And I don't know. All I can say is that these are occurring in scrapie-affected families in the flocks that I looked at. I will explain a little more in detail just what I mean by this.

Dr. Gordon:

Do these conditions not occur in nonscrapie-affected families?

Mr. Parry:

In those under my supervision, no.

Dr. Gordon:

I see. So this kind of disease occurs only in families where scrapie exists? And it does not occur in families where scrapie does not exist?

Mr. Parry:

We think it does not, but I have inadequate medical information to answer that.

Dr. Stamp:

If I may speak again, sir, the conditions which Mr. Parry describes, especially the one that he referred to as the pining condition, in my experience--and it's a very wide experience of sheep disease--this condition occurs very commonly on other farms where scrapie does not occur, in breeds where scrapie is not reported.

How does Mr. Parry distinguish pathologically or in any other way between this type of disease which I see commonly on farms where scrapie does not occur and the type of disease that he diagnoses as being characteristic of a scrapie farm?

On what basis would he differentiate these two conditions?

Mr. Parry:

I am dealing with flocks under a very high plane of nutrition, in which nutritional trace element deficiencies are most unlikely to occur. We have no reason to suspect the presence of any toxic agent. There may be. They are extremely well shepherded. There is no parasite, internal parasite, problem, partly because of the system of close-folding and the moving on of sheep.

At postmortem examination on these animals we fail to find parasites. I'm not a professional parasitologist and I may have missed them. I'm not saying there may not be some.

Dr. Gordon:

What is the histological appearance of the central nervous system in these cases? It is the same as that observed in the classical cases of scrapie?

Mr. Parry:

I'm not saying what--I'll come to that in a moment. The point is that this is a group--As I said, these are atypical ones. I'm going to now tell you in a moment that life is too short to do the pathology of all this. I'll tell you what the pathology of the classical scrapie syndromes is.

All I'm saying is that in flocks in which scrapie occurs, if you have a complete record

of all illness and particularly all neurological illness, you will sooner or later come across a small proportion of what I call atypical syndromes. We do not count them as scrapie in our data. We merely draw your attention to it because we think we would like to know more about it, and the records are being kept against the day when perhaps we can look into it.

Dr. Gordon:

So these atypical syndromes are definitely not scrapie because there are no lesions in the C.N.S?

Mr. Parry:

No. Perhaps the slides were not clear. It is only the first slide with five syndromes which is labeled "Typical Syndromes" which we consider as scrapie and which are incorporated in our epidemiological survey.

Dr. Gordon:

It's to these you are going to attach a pathology now?

Mr. Parry:

Yes.

Dr. Dickinson:

I'm sorry here. We seem to be back now to the position of Mr. Parry's presentations in his first paper in Heredity in which he amplified fully his findings and his data and his views on what was causing scrapie there. As a geneticist--I'm only a geneticist; I'm not a pathologist as Mr. Parry is--I'm put in a strange position of finding that you can only do genetics if you precisely define what you are talking about and then continue.

Mr. Parry's early work was such that it had this fringe, though, of cases which had no pathological commentary on them, which might have been scrapie or might not. And you cannot apply genetics under such circumstances.

You have got to take a stand and talk about the genetics of the stand you have taken.

And this is why there is this fuss going on around here at the moment on this particular issue. We seem to be back 3 or 4 years on this point. I'm surprised.

Mr. Parry:

I think I said, or I intended to say if I didn't, that all the detailed genetic work has been done on a very small group in which the clinical signs are typical and the pathology has been supported. And the pathology you will hear about.

Dr. Dickinson:

Scrapie is a disease of populations of sheep. Scrapie as it affects the American sheep industry and as it affects the British sheep industry is a situation affecting populations, not little groups taken out from that situation and where you find certain situations happening. The remarks that I want to make apply to attempts to sample the population, not to describe one or two peculiar instances from that population.

And if Parry is now going to speak of scrapie in certain unusual situations and not refer it back to the field situation, then to that extent he's edging off the problem which I think most of the breeders here really ought to be concerned about.

Mr. Parry:

Mr. Chairman, I think we could put off further discussion of this point until I have presented my data on how my populations have been selected. Then we can see whether they are, in fact, biased or representative.

Dr. Goode:

Yes, to satisfy this question that Dr. Stamp has raised so that we have a better understanding of the relationship of the different type of animals that are being examined. You do plan to go into more or less an outline of the examinations?

Mr. Parry:

Absolutely, Mr. Chairman. I'm well aware of the fallacies of relying on clinical diagnosis alone. And my clinical skills, such as they are, have always been built up on William Osler's technique of carrying out a pathological examination to confirm, or otherwise, my clinical diagnosis.

Dr. Stamp:

May I speak once more, sir? May we have this item next, if I might insist on it? I want

to know what Mr. Parry is talking about when he talks about scrapie.

He has, in fact, listed a number of diseases which Dr. Gordon has already mentioned. The type of farm and the type of sheep that Mr. Parry is dealing with are not exclusive to him nor to England. Similar farms occur in Scotland as well, and well-fed, well-managed, and well-shepherded sheep are found in Scotland as well as in England. Scrapie is often not present. And yet, the diseases which Mr. Parry classifies as scrapie, occur on these types of farms. I know no parasites are present. I know there are no histological lesions. I have had experience of this over the last 20 years, and I would like to know the causes of such disease.

I think, however, it is presumptuous of Mr. Parry at the present stage of knowledge to say that they have anything whatsoever to do with scrapie unless he has clear evidence of this. And if Mr. Parry has no such evidence that these diseases have to do with scrapie, either on a pathological or microbiological basis, then I suggest for the rest of the day we forget them.

Dr. Goode:

Well, as Mr. Parry has stated earlier, or at least the understanding I had of his remark was that he did plan to get into describing what evidence he has along these lines. So I am assuming that this will follow.

Mr. Parry:

That's correct.

Dr. Anderson:

Mr. Parry, I have an unrelated question. You referred to a flock there in which over a 10-year period I believe you said you had introduced 1,500 sheep.

Mr. Parry:

No, I have a breeding population of 10,000 ewes in about ten breeds, out of which over a 10-year period we have had 1,500 cases of scrapie. They involve 50-odd flocks, between 50 and 60 flocks at any time. But you will be told exactly what the turn over has been and how long they have been under supervision, because this is very important I'm sure.

Mr. Yohe:

Getting back to some of these atypical cases, I don't know what's going to come so I'm not pursuing some other course here, but when these animals died did they show brain damage of the neurons such as was described yesterday?

Mr. Parry:

Well, I will be talking about this in a moment. We haven't fully examined these pathologically. The macroscopic pathology has been done, but, as you will find in a moment, the microscopic pathology is a very involved process.

Gentlemen, if I can proceed to our concept of the pathology of this classical scrapie, that is those five syndromes on the first slide which represent at least 95 percent of all cases that we have encountered. These atypical syndromes have not been fully examined pathologically. We have done a little preliminary work, but I do not wish to speak to them because a great deal more has to be done. This may answer Dr. Stamp's point whether they are, in fact, within the scrapie family or not.

But these first five are undoubtedly scrapie by all the classical criteria, and it is to those that I will speak with regard to the pathology.

NATURAL (SPONTANEOUS) SCRAPIE IN SHEEP. II. THE PATHOLOGY OF NATURAL SCRAPIE IN SHEEP WITH PARTICULAR REFERENCE TO THE NEUROMUSCULAR SYSTEM

Elisabeth Beck,[1] P. M. Daniel,[1] and H. B. Parry[2]
(Presented by H. B. Parry)

Sheep with scrapie suffer from ataxia, tremor, weakness, wasting of skeletal muscles with rubbing, excessive thirst, instability of the heat regulatory and cardiovascular systems. Two clinical types predominate; one in which cerebellar signs predominate and one in which metabolic and autonomic disturbances are more marked.

Pathological changes, consistent with some of these clinical signs, were found in the cerebellar system and in the hypothalamo-neurohypophysial system.

The cerebellar system had severe to moderate degeneration in the cerebellum and its main afferent nuclei and pathways; efferent connections were less affected. The widespread vacuolation of nerve cells found in the brainstem nuclei of affected animals and almost exclusively confined to those nuclei which have connections with the cerebellum, is regarded as a sign of retrograde change in the cell due to degeneration of its mossy terminal within the granular layer of the cerebellum. Such degenerating terminals (mossy endings in the cerebellum, bouton terminaux in the pons) were the first indication of system degeneration.

The most common form of this degeneration of the cerebellar system was an olivo-ponto-cerebellar degeneration, but there were also cases of ponto-cerebellar degeneration and one case of spino-ponto-cerebellar degeneration. The three types occurred in the same 'family' of sheep. There are analogies between scrapie and the various forms of human hereditary cerebellar system degeneration and between scrapie and kuru.

The hypothalamo-neurohypophysial system shows degeneration within the supraoptic and paraventricular nuclei, the supraoptic-hypophysial tract and the neural lobe of the pituitary gland. This degeneration was similar in type to that found after pituitary stalk section. It is thought that degeneration within the hypothalamo-neurohypophysial system not only causes diabetes insipidus but by disturbing the balance which exists between the hypothalamus, the pituitary gland and its target organs may lead to a variety of other metabolic disturbances.

Of the 25 cases examined 24 showed a myopathy essentially of the 'polymyositis' type in representative samples of skeletal muscle.

It is of interest that the stigmata commonly associated with the more usual communicable encephalogenic virus infections - perivascular cuffing, round cell infiltration, sub-meningeal infiltrations and glial nodules - were not found.

REFERENCES

Beck, E., Daniel, P. M. and Parry, H. B. 1962. Hypothalamic and Cerebellar System Degeneration in Sheep with 'Scrapie'. Proc. IV Int. Congr. Neuropath., Munich, 3, 269-274.

Beck, E., Daniel, P. M. and Parry, H. B. 1964. Degeneration of the Cerebellar and Hypothalamo-neurohypophysial systems in sheep with 'Scrapie'. Brain (Lond.), 87, 153-176.

[1] Department of Neuropathology, Institute of Psychiatry, Maudsley Hospital, London, England.
[2] Nuffield Institute for Medical Research, University of Oxford, Oxford, England.

DISCUSSION

Dr. Goode:

Dr. Parry, if I may make one remark at this point--and I hope my question is not an unfair one--1 begin to realize a little more fully what was actually behind Dr. Stamp's earlier question.

It is certainly interesting, your remarks regarding your observations, concerning the lesions and all that is involved here. I think it becomes more important as we go along that since the reference is made to scrapie-infected sheep that the criteria for distinguishing them as scrapie infected--

Mr. Parry:

Affected.

Dr. Goode:

Affected. Yes.--could be alluded to, and possibly if there is any method of, we might say, rechecking as to this situation. I hope this is not an unfair question.

Mr. Parry:

No, not at all, I shall proceed. First of all, 1 must make it clear I'm not concerned with the general diagnosis of scrapie. 1'm only concerned with putting a diagnosis on neurological illness in those groups of flocks with which 1 am concerned.

They represent the leading flocks of ten breeds at least. But in point of fact we have only substantial numbers in four breeds, Suffolks, Dorsets and Polled Dorsets, and also Hampshires.

Now, to be effective as an instrument for determining epidemiological data, in our view you want a closed breeding population or a nearly closed breeding population. It's easy enough to insure that no females are brought in.

The second thing is that as far as possible, rams are bought only from other flocks in the group, in what we call our breeder groups. We have two Suffolk breeder groups because we have so many. We have a Polled Dorset and Dorset Horned group and a Hampshire breeder group. When we have a breeder group we like to have at least ten flocks with about 1,500 to 2,000 ewes who will adopt a common breeding policy and who will, by and large, only buy their rams from within the group so that we know about them.

In the last 12 months we have got to a stage in the most advanced breeder group when we can put this firmly into practice. Until now we haven't been able to say to a man, "No, you mustn't go and buy a ram from Mr. Jones who is not in the group," because he said, "It's got a wonderful head, you know. It will win at the Royal Show," or something. And therefore he has to be allowed to do it because this is of economic importance. And we have to keep the goose that lays the golden eggs, as far as 1'm concerned, happy. I'll be talking about that more.

1 am only concerned with the recognition of scrapie within this selected population. I may be asked to give an opinion outside. 1 do it with great reluctance. 1 will never do it unless 1 see the animal alive or it is brought to me and I clinically examine it before post-mortem examination and I do the post mortem myself, and we do the pathology on it. If we can get pedigree data, so much the better.

This is one of the difficulties with outside flocks. We don't know.

Now, within my breeder groups we have absolute identification, so we have a complete pedigree, and a life history of the sheep to start with. We have a clinical examination by myself. We have the pathology.

The gross pathology is done by me or by a colleague and 1 see it. And the microscopic pathology is done by my medical colleagues.

And all three have got to add up to give us a diagnosis of scrapie. That is, if we have what looks like possibly scrapie and the pedigree is such that it is virtually impossible or unlikely, we go back again and look at the histology in more detail.

To date we have never had an instance in our animals in which there has been conflict between the microscopic pathology, the clinical examination, and the pedigree. And we require data on all three.

1 would further add that in our diagnosis we go on the macroscopic pathology. That is, there should be muscle lesions. It's not always apparent, particularly in particular cases in which muscle wasting is not a main consideration. And we go on endocrine changes.

In nearly all of these the thyroids are of less weight than they ought to be. And we

know this from an extensive series of observations on normal sheep at various times of the year and of pregnancy. We always weigh all the endocrine organs--in fact, all the target organs in the body.

You may say, "What is the normal range for thyroid or adrenal or pituitary?"

Thyroids I can say categorically are about half the normal size. In Suffolks they ought to be, under English conditions, between four and six grams. They are usually about between two and four grams.

The adrenals may or may not be enlarged. I won't go into that. This is related to the amount of stress prior to the death.

And the pituitary almost certainly is wrong, because many of them are large, that is, in excess of two grams.

Don't hold me down, Dr. Hadlow, too tightly about this, because I will give you such information as I have later. I didn't prepare this, and I don't know how to interpret pituitary autopsy weights. There is obviously great variability.

But the point I want to make is that in our view there is a disturbance, a general disturbance, in the target organs of the pituitary gland. Details I can't tell you, gentlemen. We haven't worked them out.

The last and important point which I thought we took for granted is that in all these cases of what we think are genetically determined natural scrapie in which we have done transmission experiments we have demonstrated the presence of a transmissible agent.

That is, we are perfectly happy there is a transmissible agent as far as we know.

Dr. Dickinson:

Could we have the number of cases you have transmitted it from?

Dr. Goode:

I was going to ask if routinely you conduct or you apply the techniques associated with transmissibility of this by one method or another as a matter of confirmation of findings.

Mr. Parry:

No, sir, we don't. It is physically quite impossible. I haven't the resources of Dr. Gordon and his animal compound. This is quite impracticable.

All we can do is to take brain specimens--as we have done in about ten instances--and inoculate sheep intracerebrally--and we are now inoculating mice, which raises another point--with scrapie brain material from Suffolks. It takes a very long time in mice on primary inoculation.

Dr. Gordon:

What about the pathology of the experimentally produced disease by inoculation? Does this match exactly the natural disease, or do you find some difference?

Mr. Parry:

I'll speak to that later, Dr. Gordon. It's on the program. And I firmly talk only about the natural disease--

Dr. Gordon:

Would you say quite shortly whether the histopathology is the same or different?

Mr. Parry:

I will say it's very similar.

Dr. Stamp:

May I speak again, sir? I'm sorry to keep speaking, gentlemen, but I feel we have got to get these things right before we begin today. I have no question. These are observations.

The criteria for the diagnosis of scrapie are obviously very complex, and if I may go through them I would like to make a few comments.

If I could take the pathology to begin with which Mr. Parry has described--And I might say that I cannot have the responsibility passed back to Professor Daniel--Mr. Parry is giving the paper and therefore, must accept the responsibility for what he says, and therefore, he must answer the questions.

Beginning with the muscle lesion which you so beautifully showed on your slide, Dr. Hulland and myself working at The Moredun Institute, using Suffolk, Cheviot, Hampshire, Halfbred sheep, Border Leicester sheep, probably several other breeds as well, carried out a very exhaustive examination of the muscle of normal and of scrapie sheep.

We have quite experienced pathologists at Moredun, particularly so, as far as sheep are concerned. We examined the sheep just referred to--scrapie and nonscrapie sheep--for a whole year and never once did we find anything like the muscle lesion that Mr. Parry showed in the photograph on the screen. Not once did we find such a lesion.

I would insist that this morning we know what muscle lesions are found by Mr. Parry in scrapie. Mr. Parry and his colleagues have been so positive in the past that I think they should be positive this morning.

In the paper this morning, for instance, Mr. Parry said that they did not deny that brain lesions were present in scrapie. If I may refer him to the article in the VETERINARY RECORD, he was pretty scathing about veterinary pathology and the lesions in the brain. If he hadn't looked at the brains then he shouldn't have been so positive.

The next point is that not only did we examine the muscles of these sheep macroscopically, we also examined them microscopically, and we did find some microscopic lesions but they were in no way different from the type of lesions one finds in other wasting conditions and in other conditions where one gets incoordination. The type of muscle lesions that one might expect with a damaged central nervous system are, in fact, secondary lesions.

In the first article published in the LANCET, the muscle lesions described were not secondary lesions in the muscle. They were primary lesions that were described.

I will go on a year now and during the following year, Dr. Hulland, myself, Dr. Zlotnick and Mr. Rennie examined I think in the region of six or seven hundred sheep which came in to the laboratory for general diagnosis, either for scrapie or diagnosis of other diseases. We were so interested in these muscle lesions that we examined the muscles of all these sheep, both macroscopically and microscopically. Much to our astonishment this year we found such muscle lesions--but not only in scrapie sheep. We found them in every kind of diseased sheep that came in to the post-mortem room.

I would suggest to you and I would suggest to Mr. Parry that such lesions are probably nutritional in origin and have nothing to do with scrapie. They most certainly are not confined to scrapie sheep.

Following this particular year we have examined the muscles I should think of a thousand sheep by now and we have not seen these muscle lesions again.

The next point I would like to make is regarding the brain pathology which has been so nicely described this morning by Mr. Parry. He said that the examinations had been confined to three breeds of sheep--Suffolk, Dorsets. I would like to know what the third one was, if you don't mind, Mr. Parry, because this is important to my argument.

Mr. Parry:

Hampshires. We have no experience with Scottish breeds.

Dr. Stamp:

No, but we also keep Suffolks in Scotland too.

The microscopic brain pathology of scrapie in the Cheviot breed, which is the type of sheep that we examine quite commonly in Scotland, is in many ways dissimilar to the pathology we see in scrapie in Suffolk sheep. In Cheviot sheep lesions in the cerebellum are absent except in about 20 percent of the cases, and most certainly lesions are not present in the hypothalamic area.

The majority of lesions in the Cheviot sheep are confined to the medulla and the more posterior areas of the brain.

The lesions in Suffolk sheep are more extensive and lesions are often present in the hypothalamus and also in the cerebellum. The lesions are the lesions of status spongiosus, astrocytosis, not very marked, and considerable vacuolization.

If the hypothalamic lesions and cerebellar lesions are so important as the primary lesions of scrapie, then all I can suggest to Mr. Parry is that scrapie in the Cheviot sheep must be entirely different from scrapie in the Suffolk sheep, because these lesions do not occur in Cheviot sheep.

And if Mr. Parry's diagnosis of scrapie in general depends upon the demonstration of such lesions, then if he came to Moredun I would suggest he would misdiagnose at least 80 percent of our cases of scrapie.

So as far as the thyroid gland and the pituitary gland and the adrenal gland are concerned, I would agree that one does or does not get alterations depending upon the stage of the disease at which the animal is examined. I would also suggest one can get similar variation in a multitude of other stress diseases. They are, in fact, not specific to scrapie and therefore are not diagnostic of scrapie.

I still, Mr. Chairman, am not at all clear what are the criteria of scrapie as applied by Mr. Parry to his material. If they are what he has said this morning, then I would say he would miss 80 percent or more of the cases of scrapie as we see them at Moredun.

Mr. Parry:

I have no experience with Cheviot sheep. It's very interesting what you say.

Dr. Stamp:

I'm sorry, sir, if I may interrupt. What I have said is not confined to Cheviot sheep. You can get all gradations of lesions from Cheviots, Hampshires, or Suffolks. Half-breeds (being about halfway between the Cheviot, the Suffolk), North Indian sheep, and Swaledales. The scrapie sheep from North India in which muscle lesions were not present had typical vacuolations in the medulla but not the lesions described by Mr. Parry. These lesions are not common in anything except possibly the Suffolk and I think one form of scrapie in the goat.

Mr. Parry:

Do your Cheviot sheep show clinical signs of disturbance of water intake, cardiovascular regulation, and so on?

Dr. Stamp:

I have no idea whether they show these physiological signs. All I can say is they show typical classical signs of scrapie as you described them on the board this morning.

We accept exactly the clinical criteria you accepted in your first table. I cannot be sidetracked by what you find in the way of physiological disturbance. We must stick to what you accept as your clinical criteria.

The sheep I have seen showed typical clinical signs of scrapie as stated by you on the board this morning. There is not the slightest shadow of doubt that the scrapie you described and the scrapie I am describing are indistinguishable except in a matter of possible emphasis and detail.

Mr. Parry:

I would doubt that. The point is--

Dr. Stamp:

On what grounds, sir, would Mr. Parry doubt it?

Mr. Parry:

Because there is wide variation even in our material in the degree of disturbances in these homeostatic mechanisms. And some sheep do not have them. The slide had to be simplified because I can't put all the points on a slide. It overburdens it. I can only put the main headings.

We know what we get in the material that we have lesions in the medulla and the cerebellum--that is, in the afferent motor pathway, the cerebellum, in those sheep which show ataxia.

In those sheep which don't show much disturbance in water regulation and cardiovascular instability and temperature regulation, if you haven't looked for these you wouldn't know whether they are there or not, and I missed them for long enough. But having--

Dr. Stamp:

Mr. Chairman, may I interrupt sir? We want to know what are the criteria of scrapie. If they are the homeostatic mechanisms, let us know that they are and let us finish there.

Mr. Parry has given 10 minutes' discussion to how he diagnosed scrapie, and the listed clinical syndromes on the board were, in fact, quite typical of what we recognize as scrapie. I'm not interested in homeostatic mechanisms. If they are, in fact, specific for scrapie, let him base his diagnosis on these things and on nothing else.

We want to know what your ratios are based upon that you are going to bring out today.

Dr. Goode:

In other words, Dr. Stamp, if the criteria that were stated early in the presentation are those upon which scrapie is diagnosed, this

puts us at one point of understanding, and you would like to have a relationship of this type of animal or at least this type of recognition of signs related to the data as it will be presented later on.

Am I stating this correctly?

Dr. Stamp:

You have put it much more clearly than I have done, sir.

Dr. Goode:

Will this be the case as the seminar is developed here this morning, Mr. Parry?

Mr. Parry:

Indeed, the main, obvious clinical signs which anybody can see were put on the slide. The more subtle clinical signs which you can elicit by looking in the right way substantiate what I already put on the board. That is point one.

And the clinical diagnosis depends on a consideration of the whole. I come back to that point, Mr. Chairman.

Secondly, the pathological confirmation, if you like, is that there are vacuolated neurons.

Dr. Stamp:

Thank you. That is all I wanted to know.

Mr. Parry:

Except--except--in certain very early cases in which we have other evidence either by the demonstration of a transmissible agent or on pedigree.

Dr. Stamp:

No, sir. I object to this dependence upon pedigree. This pedigree argument is prejudging the situation.

Mr. Parry is having a circular argument here. He wants to prove, first of all, that the pedigree is right by the pathology, and then he proves the pathology right by the pedigree. He cannot have it both ways.

Mr. Parry:

No, Dr. Stamp, scrapie is not a simple situation.

Dr. Stamp:

I couldn't agree more with you but we cannot have circular arguments. You either have a diagnosis of scrapie based upon some specific lesion or some specific signs, which we want to know. You have already said that it is based upon vacuolation of the medulla or is it not?

Mr. Parry:

Now, vacuolation of neurons is not specific to scrapie, gentlemen. It can occur in a number of anoxic and experimental conditions. We have seen it in cases of tetanus in man.

It is probably, we think, merely indicative of neuronal death and transneuronal degeneration.

True, in scrapie this is much more frequent and much more massive than in any other neurological illness of sheep of which we have any knowledge.

But I must take issue with this business of making a diagnosis of any disease state, particularly those in which there may be a hereditary or genetic element on a single factor--You have to take into account the whole situation. I will fight to the last this concept that the laboratory man should be able to make a diagnosis alone.

You take the whole field, gentlemen, the epidemiology, the pedigree. It doesn't prejudice what the inheritance is. It's a pedigree. It's a point of fact. The clinical signs. And the course of the disease. The gross pathology. Any function test you may have been able to apply if you have the facilities and the patience. The histopathology. And you take all these into consideration.

And as far as our experience is concerned and as far as the data which will be presented in our crucial work about a possible hereditary mechanism, all these criteria have been met, and it doesn't depend on the pedigree. It depends on a consideration of the history of the flock and the breed, very accurate history I might state, the clinical signs and the pathology I have mentioned.

And wherever we have been able to we have confirmed that there is a transmissible agent. But life is too short to do it every time.

Dr. Goode:

Well, I think really the point that would help to clarify the situation possibly would be a determination of the significance that is placed on any or all of these with regard to the

diagnosis of scrapie or the classification of an animal as having scrapie. This is the way I understand the situation personally.

Mr. Parry:

They must all be positive, Mr. Chairman, and they must all fit into the pattern that I as a biologist in the widest sense consider the diagnosis, and if there is an error it is observer error in me, a constant error in the main.

Dr. Goode:

I don't think any of us would disagree with the desirability of being as comprehensive as possible in the observation and determination of all these factors. I think we all appreciate the fact that you are bringing this type of information before us.

There seems to be this narrow line of demarcation--what we would all completely understand as a positive scrapie as opposed to one that may not be a clearcut situation.

Dr. Gordon:

Mr. Chairman, I should like to ask Mr. Parry if after listening to Dr. McDaniel yesterday he would agree that Dr. McDaniel was able to disgnose scrapie on the histological evidence he presented.

Mr. Parry:

I would say I wouldn't attempt to, Dr. Gordon, because I would not attempt to do so unless I had seen the animal alive and I knew the flock and the breed from which it came.

But then I am in a specially privileged position. I am not called upon to give an opinion unless I want to. That is, I am only giving opinion on those cases which are within our supervised groups.

Dr. Dickinson:

Could I ask one simple question? As I said before, I am a geneticist and not a pathologist, but I am a little lost, so can we put a simple question which has a yes or no answer to it in this situation?

Have you diagnosed as scrapie an animal which did not have neuronal vacuolation?

Mr. Parry:

Yes.

Dr. Dickinson:

Thank you.

Mr. Parry:

Yes, because--Allow me. Because it had the very early signs of degeneration of the mossy fibers.

Dr. Dickinson:

How have you shown that these are absolutely specific--absolutely specific--to scrapie, and how many non scrapie animals have you observed in this particular respect, from which breeds, from which farms, to establish the specificity of this particular sign? Unless we are prepared to talk in terms of objective criteria, not vague things that are going to be published uncontrolled at some infinitely indefinite time.

Mr. Parry:

The point is that amongst neuropathologists--I am only an amateur at this--

Dr. Stamp:

Sir, this is a paper on neuropathology, and we must stick to this.

Mr. Parry:

Yes.

Dr. Stamp:

May I ask a complementary question to Dr. Dickinson's? Has Mr. Parry diagnosed scrapie when muscle lesions as he has shown in his photograph today were not present?

Mr. Parry:

Certainly.

Dr. Stamp:

You said a moment ago, that every criterion that you have given this morning must be present before you diagnose scrapie. You have now said exactly the opposite.

Mr. Parry:

Dr. Stamp, I told you that the 25 cases of the last series which are just being published--

Dr. Stamp:
I am not interested in these special 25 cases. I am interested in every case of scrapie that is going to be quoted today in connection with your genetic ratios.

Mr. Parry:
I said that of the 25 cases in which muscle lesion had been diagnosed macroscopically they were confirmed in 24. One is fallible. These are not absolute. And this raises another point, gentlemen--

Dr. Stamp:
I am quite clear that when muscle lesions are present macroscopically you see them microscopically. I haven't the slightest doubt about it. What I want to know is if scrapie is diagnosed in the absence of macroscopic lesions in the muscle.

Mr. Parry:
Certainly they are, if the other lesions are present.

Dr. Stamp:
What others?

Mr. Parry:
The brain lesions which I--

Dr. Stamp:
These particular lesions you're talking about?

Mr. Parry:
Yes.

Dr. Stamp:
The cerebellum and mossy fibers and so on?

Mr. Parry:
Yes.

Dr. Stamp:
Nothing else? No other brain lesions except these?

Mr. Parry:
Oh, there are other lesions.

Dr. Stamp:
No, I want to know, I must know what these criteria are. It's no good Mr. Parry saying, "Oh, no, there are others." I want to know the criteria.

Mr. Parry:
I think you well know there are many other lesions, or at least there are changes in certain neurons.

Dr. Stamp:
Yes, I know this. Adrenals are increased in size in many animals. Does Mr. Parry diagnose this as scrapie?

You can't pass from one thing to another because one thing is present and half a dozen things are absent. This isn't diagnosis based upon pathology as I understand it.

Mr. Parry:
Many of these things are not specific for scrapie. This degeneration of mossy fibers, and Vacuolation is prominent in scrapie. It occurs in other syndromes, obviously.

Dr. Stamp:
In what other syndromes in sheep has Mr. Parry seen excess vacuolation?

Mr. Parry:
Not in sheep.

Dr. Stamp:
I want to know in sheep. I'm not interested in what happens in human beings that take overdoses of alcohol and one thing and another. I want to know what happens in sheep. In sheep only. What other conditions have you seen vacuolated neurons in numbers?

Mr. Parry:
We're not disputing this, Dr. Stamp.

Dr. Stamp:
You have been disputing it all morning as far as I can see. Do you accept in scrapie, in all cases of your scrapie, you get an excess of vacuolated neurons? This is all I want to know.

Mr. Parry:
In the great majority, yes. Wait a minute.

Dr. Stamp:
I want to know if in every case you accept this as a method of diagnosis as given by

Dr. McDaniel yesterday. It's a simple question. Yes or no?

Dr. Goode:

In other words, do you confirm a diagnosis on this single observation?

Mr. Parry:

No, I would not, because I would take the whole--

Dr. Stamp:

If, in fact, you don't accept this, I want to know what criteria you do, in fact, accept.

Mr. Parry:

There are vacuolated neurons in all the material which will be included in the paper on the genetics--This is I think perhaps what you want me to say.

Dr. Stamp:

You do, in fact, accept excess vacuolated neurons when present as being a method of diagnosis.

Mr. Parry:

As an aid to diagnosis I do.

Dr. Stamp:

Thank you, sir. That's all I want to know. You use it as an aid to diagnosis.

Mr. Parry:

You see, I don't take the simple view that any one of these criteria is necessarily absolute.

Dr. Stamp:

Neither do we. But if you don't take the simple view you end up in a morass of uncertainty, and this is what worries me.

Mr. Parry:

Of course, that depends on how you do the work, you know, Dr. Stamp.

Dr. Stamp:

Not when you're quoting specific genetic ratios it doesn't.

Mr. Parry:

Mr. Chairman, I'm not sure this can be very profitably taken any further. My view is you have to take the broad biological view, the broad biologist's view of this particular disease and not any single narrow one criterion.

I realize that I can do this. I appreciate that others may be in a position where it is difficult.

We take all the factors I have discussed into consideration.

If anybody would like to cross-question me further in private I shall be only too happy to, but we are getting very behind.

Dr. Goode:

There is one comment that I wanted to make again. I have discussed this matter with Mr. Parry during the break there, and that is the fact that there has been some criticism regarding the lack of parliamentary procedure, so to speak, here from me, the chairman of this meeting, and I wanted to reiterate again the fact that Mr. Parry had insisted that we agree that there would be an open discussion and debate, to use his own terms, on this.

There is one request that I would like to make, though, and that is that in the comments, in the questions and other parts of the discussion, that the person that is speaking be extended the privilege to go ahead and finish his remarks before he is interrupted. And if he could have your cooperation in this, I would certainly appreciate it. I know Mr. Parry would also.

And the reporter said she will appreciate it more than anyone else.

Well, we will proceed then Mr. Parry.

Mr. Parry:

First of all, if I may be permitted just to refer back to Dr. Stamp's comment because I fancy we have been at cross purposes a bit, which I didn't realize.

There are two points I want to make. I was trying to make a general pathological point that the type of lesion that you get in the CNS, all muscles, and all the endocrine systems is not necessarily pathognomonic of any one disease, but they represent a type of reaction of that part of the body to numerous different situations.

But in scrapie, the lesions are so massive that they can be taken, as far as we know at present, for practical purposes as

pathognomonic. I think this is what Dr. Stamp is getting at, if not--

This brings me to the second point. A question of the diagnosis as it applies to the data that we are going to present, the detailed epidemiological data particularly that which my colleague, Mr. Draper, will present about the possible hereditary component.

Every case meets the simple, clinical criteria shown on the first slide, the five classical scrapies, and every case has been confirmed with the simple criteria of vacuolation in the CNS, and we have not used pedigrees at all.

May I ask Dr. Stamp, Mr. Chairman, whether that does in fact meet what he was really trying to get at?

Dr. Stamp:

Yes.

Paper No. 10

NATURAL SCRAPIE OF SHEEP: ITS OCCURRENCE AND DISSEMINATION IN THE FIELD. I. THE NATURE AND RELEVANCE OF EVIDENCE

H. B. Parry[1]

Vital statistics of biological phenomena as they occur in the field can be quite as important as laboratory studies in providing relevant data for scientific evaluation, provided certain precautions are taken to ensure the accuracy of the observations. In the case of a condition such as scrapie, in which sociological and economic considerations may distort the reliability of the witnesses, it becomes essential to take precautions to ensure that the data are entirely trustworthy and complete.

Ten years' experience of close collaboration between practical sheepmen and research scientists allows one to lay down certain rules for British conditions, which we know must be met if the data are to conform to the highest scientific accuracy.

GENERAL PREREQUISITES FOR ENSURING RELIABILITY OF FIELD DATA

1. Permanent individual identification.
2. Accurate written records of matings, lambings, identification marks, etc.
3. Independent supervision through at least bi-annual visits.
4. Comprehensive health records of all neurological illness.
5. Continuity with stability of farm organisation over a decade.
6. Semi-closed or closed breeding populations.
7. Absence of distorting biases.

IDENTIFICATION

There should be at least two and preferably three permanent tamper proof systems with temporary fleece numbering (except in longwools).

REQUIREMENTS FOR PROVIDING ACCURATE WRITTEN RECORDS

1. Close supervision by an independent scientific investigator.
2. Mutual trust and respect between stockman and investigator.

[1] Nuffield Institute for Medical Research, University of Oxford, Oxford, England.

3. A sense of personal responsibility to each other by both parties.

4. A mutually agreed code of basic management practice and recording incorporating in-built checks.

5. A suitable simple form of recording notebook and register.

6. Strict adherence to the code with written records.

7. Immediate recording of all breeding and health records.

8. Make the suppressing of information personally disadvantageous.

9. Guarantee absolute confidentiality of all information.

10. Eliminate any element of competitiveness.

11. Ensure the long-term pecuniary advantage to stockmen.

12. Enlist the intellectual interest of the stockmen.

These requirements have been met in the case of Class 3 B & C members of the British Sheep Society; other junior members are grading up to these standards.

COMPREHENSIVE HEALTH RECORDS

These are essential:

1. To show up cases of undiagnosed neurological illness and illthrift, which may be atypical manifestations of scrapie, as well as obvious cases of scrapie.

2. To reveal the nature and extent of other disease or illthrift, which may be causing 'competing risks' to be large. Normally wastage from these are 2.5 percent of the ewe flock p.a.

FARM STABILITY

It is essential that the farm is a strongly viable economic unit under competent management and with an adequate labour force in emergencies. Normally any management over 50 years of age must have an obvious successor in line.

SEMI-CLOSED AND CLOSED BREEDING POPULATIONS

One aims to form breeder groups each of which forms a closed breeding population with about 2,000 breeding females. These breed their female flock replacements and do not go outside the group for sires, except under exceptional circumstances.

In each breeder group a joint breeding policy is adopted whereby certain economic objectives are given priority as soon as, or concurrent with, any disease control programme. These groups are endeavouring to control and largely eliminate hereditary defects as well as the common microbiological and parasitic diseases, while building up inbred strains of improved sheep, whose production characteristics are available from the supervised authenticated records.

DISTORTING BIASES

1. Lack of completeness and impartiality of stockmens' evidence: suppressing evidence is not to their advantage.

2. Losses from competing disease risks: strict control measures for other diseases and causes of flock wastage.

3. Losses from slaughter and quarantine policies: these are absent in Britain.

4. Variations in breeders' selection and culling policies: these are small and infrequent outside our attempts to minimise losses from scrapie.

CONCLUSIONS

It has proved practicable under British conditions to build up a voluntary, largely self-financing organisation (now the Sheep Development Association and the British Sheep Society), which has been able to meet the above requirements. Their senior flocks provide records of the requisite accuracy.

REFERENCES

The Sheep Development Association Limited and British Sheep Society. Introductory Note, July 1963. The Sheep Development Association Limited, Holton Paddocks, Wheatley, Oxford, England.

Rules of the British Sheep Society, July, 1963. The British Sheep Society, Holton Paddocks, Wheatley, Oxford, England.

Dr. Dickinson:

You say you use the ABRO system of recording. So do we. What do you estimate your error rate in this system is?

Mr. Parry:

I don't know.

Dr. Dickinson:

We have been rather surprised. I don't want to quote any figures but all I am saying, now the blood typing in sheep is coming along, unbelievable mysteries emerge. I am not talking about scrapie work here. The Animal Breeding Research Organization record system to which Mr. Parry referred is one that is instituted on farms where we are selecting for this or that growth or wool characteristics, et cetera, and it horrifies us what can happen to this kind of a farm situation in terms of unattached progeny, how this progeny which on all objective evidence was the progeny of this ewe but cannot possibly have been in terms of blood typing evidence. These are mysteries which Parry has to contend with, we have to contend with, but it is an unfortunately high rate.

Mr. Parry:

I didn't mean to say that we actually used your system, but when I went to Stanhope I realized the system they were using there--

Dr. Dickinson:

It is just a fairly comprehensive reporting system.

Mr. Parry:

We had arrived at much the same as you. I know roughly what I have been told your error is. I suspect ours is much the same; we have no way of measuring it, you see, at the moment.

Dr. Burger:

Mr. Parry, when it goes to sheep, I confess I am a layman as compared to you, but I have one question. You were talking about biases. Now, there is one way; I mean, this is done with veterinary schools and other schools, if I have the slightest doubt that in a subjective judgment that a bias may enter, I may take ten of my colleagues, you know, give them twenty cases which are positive or negative, according to my criteria, and let them judge, and then I get an error of judgment, because those ten colleagues perhaps may not judge those twenty cases the same way as I will do.

Mr. Parry:

In the case of scrapie.

Dr. Burger:

I am not talking about scrapie. Any scientific object.

Mr. Parry:

A generally observer error. Yes, there is bound to be observer error in the main body of our data, because as I explained, part of this is based on shepherds, but it is the same shepherds. All the critical work which Mr. Draper is going to report is observation by me. It is my observer error and you can say it is as big as you like. I can't dispute with you.

Dr. McDaniel:

This is probably insignificant to the rest of the group, but I would like to have you comment on the Holzer stained slides that you showed, and you mentioned this to show the gliosis, glial fiber proliferation, and so forth. Actually, isn't the Holzer stain a stain for myelinated and unmyelinated tracts and fibers as well as connective tissue; is that correct?

Mr. Parry:

Not in this particular technique. It is specific for new glial fibers.

Dr. McDaniel:

Thank you.

Mr. Parry:

It is very tricky. If you kill a sheep you want to let it lie on the post-mortem table from 4 to 6 hours before you take the brain out, to get the best stain reaction. When we started wishing to employ the best technique, we immediately took the brain out, so it would be fresh, and the staining reactions were difficult. We discovered that if the sheep had been left about 6 hours, the brains stain much better.

Dr. Goode:

Dr. Wells, from Canada, has spoken to me and wishes to make a remark or two. Would you care to make your remarks now, Dr. Wells?

Dr. Wells:

Mr. Chairman, there is no need for my remark now. I was concerned with the earlier discussion and I wanted to point out that the discussion which had taken place between Mr. Parry, and Dr. Gordon, and Dr. Stamp, was not any mere academic or frivolous discussion. It was in fact crucial to this whole meeting which is not solely designed for the elucidation of scientific facts, but is leading on to control.

I was concerned that we have a basic understanding that this discussion was crucial so that when we come to control, I wanted to know whether in fact we were dealing with one and the same disease. So I merely wanted to say that I took the liberty of speaking to Mr. Parry and to Dr. Stamp during the interval, to clear in my own mind the purpose of this discussion which was taking place. I think we come to an agreement now, when we diagnose scrapie on the North American continent, according to Dr. McDaniel's standards and according to Dr. Plummer's in our animal research organization, and that we are in fact dealing with the same disease that is being discussed by Dr. Stamp, Dr. Gordon and Mr. Parry. This, I believe, has now been cleared up certainly to my satisfaction and they now agree that the criteria for diagnosis is the same.

Paper No. 11

NATURAL SCRAPIE OF SHEEP: ITS OCCURRENCE AND DISSEMINATION IN THE FIELD. II. THE GENERAL PATTERN OF OCCURRENCE IN PARTS OF GREAT BRITAIN

H. B. Parry[1]

Data on the general incidence and the definition of terms have been presented in Paper 8. The evidence is limited to a small section of the flocks in Great Britain known to the Oxford team. The disease has never been notifiable in Great Britain and no national statistics are available. As the incidence of the recognisable disease fluctuates so widely even within affected flocks and breeds over periods of a few years, added to which the disease often goes unrecognised by inexperienced (or too experienced) sheepmen, it is quite impossible to form any sort of reliable quantitative estimate of the general position. Suffice to record the pattern as revealed in a sample of the flocks, for which accurate information is available, mainly for the period 1953-64.

HISTORICAL

The earlier evidence has been well summarised by M'Gowan (1914). The disease was prevalent in the Eastern Counties of England about 1750 (House of Commons, Reports, 1955). It was rampant in the Southern Counties abutting on Dorset from 1800 - 1820, a period covering the original Australian importation into New South Wales by MacArthur of Spanish merino sheep from King George III's flock in this area. The disease waxed and waned in various parts of Britain through the 19th century during the period when many North American foundation importations were made; it was serious enough in the Border Counties in the early 1900's for M'Gowan (1914) to be

[1] Nuffield Institute for Medical Research, University of Oxford, Oxford, England.

asked to carry out a special investigation. It seems to have been infrequent in Eastern England until the 1930's, since when it has become well recognised. However, in southern areas severely affected during parts of the 19th century, the disease is now quite unknown to the present generation, who had never heard of scrapie, until the recent renewal of public interest. On the other hand, the disease is now recognised among several hill breeds, which had been alleged to be absolutely free.

The information recorded about the disease is in general quite inadequate to allow any precise study of the means by which the disease is disseminated, and, indeed it allows ample scope for varying interpretations as an infectious or hereditary disease according to personal predilection. It is only in the last decade that authenticated records from a small sample of supervised flocks have allowed a detailed epidemiological study of the disease within breeder groups of 4 or so lowland breeds (Parry, 1962).

RECENT

1. Contact Between Affected and Unaffected Flock-groups

On 3 farms with high incidence flocks, other sheep of flock-groups believed to be free of scrapie, have been kept alongside and often intermingled with the affected flocks for at least 5 years. One contact flock was 110 recorded pedigree sheep retaining some of its females for breeding, and 2 were non-recorded commercial flocks of 150 and 500 ewes not breeding their flock replacements. No case of scrapie has been observed in two flocks, but one case has appeared in the 150 ewe flock in a cross between breeds now known to suffer from scrapie.

On one farm with a high incidence flock disposed of on account of scrapie, several hundred breeding sheep from a scrapie-free flock-group were introduced onto the same fields, some before all the affected flock were removed; no case of scrapie has occurred in the newly introduced sheep in 8 years.

A flock of 400 ewes acquired by purchase from 3 source-flocks and using several rams developing scrapie during or shortly after the communal mating, had cases of scrapie confined to the females and their progeny from one source-flock, which, it was learnt subsequently, had been dispersed on account of the high incidence of scrapie.

Evidence in the literature purporting to support mediate contagion is now inadmissible owing to the inadequate knowledge of the genotypes of the sheep. More adequately controlled evidence is required; studies on the introduction of animals of low scrapie frequency into high scrapie frequency flocks have been in hand for 4 years without any signs of contagion. (See Paper 12).

Contact by Coitus (These data are included in Paper 12).

In two flocks of low presumptive gene frequency on 3 occasions rams developing scrapie within 1 - 3 months of the mating season have been used. No cases have occurred in the flock ewes mated by them up to 3 years thereafter.

In an experiment using 70 animals of known genotype, non-ss rams and ss rams affected at the time of mating have been mated with non-ss and ss ewes, many of which were affected at the time of mating. After $3\frac{1}{2}$ years no case of scrapie has yet developed in any animal not of presumptive ss genotype.

We have found no evidence from our general field observations that scrapie is spread by coitus except through the genetic apparatus.

Contact During Gestation and Suckling

Adequate records are available for 23 animals whose dams manifested scrapie during their late gestation and/or early suckling. The results are given in Figure 1, the closeness of fit between the observed and expected figures is excellent, although the sample is small. They lend no support to the idea that the manifestation of scrapie during a ewe's gestation and lactation renders her offspring of that pregnancy more liable to develop scrapie. This conclusion is supported by a little evidence relating to lambs of affected ewes reared on unaffected foster-mothers; in the two instances recorded the fostered offspring have been unaffected.

Manifestation among 23 daughters born of "ss" ewes affected at the time of their gestation and suckling

Type of Mating		Progeny		
Sire	Dam	Unaffected	Affected	
			observed	*expected*
ss × *ss*		2	10	*10·8*
Ss × *ss*		6	5	*4·95*

Figure 1

Birth Order

As certain neurological disorders in man appear to be more frequent among later-born children, the birth order of 111 affected females in flock A has been examined.

For the present purpose the birth order has been taken to be synonymous with the parity of the dam at the time the affected animal was born. This is not strictly correct, since the birth order should take account of all the lambs born in gestations previous to the one in which the individual is born. However, for our present purpose this approximation will suffice.

Figure 2 shows that affected animals may be born at any period of a ewe's reproductive life. A higher proportion of the offspring of the younger ewes than of the older ewes are affected. If an infection were involved, one would expect these proportions to be similar, or even to be higher among the progeny of the older ewes. This higher susceptibility with lower parity is due to the selective mortality of ss ewes in the age-groups up to $4\frac{1}{2}$ years, so that the frequency of the scrapie gene among older ewes will be lower than among the younger ewes.

These results are consistent with other data which also indicates that birth order does not influence directly the manifestation of scrapie.

Gestational Twins

It is generally considered that identical twins are rare sheep; they are certainly very rarely detected at birth. In flock B for the

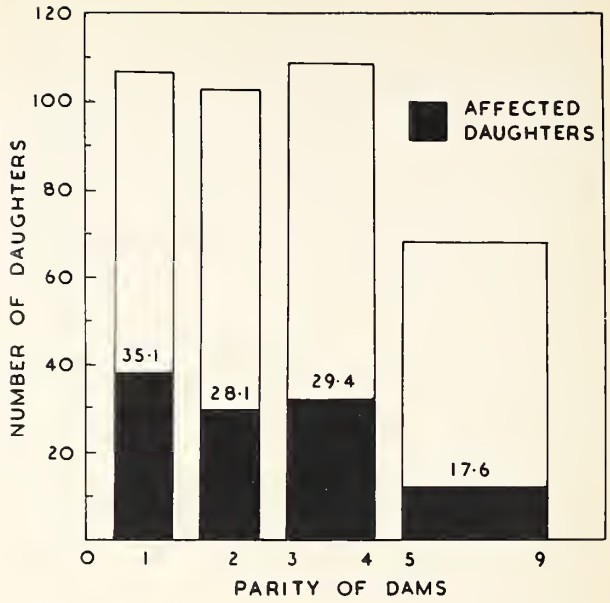


Figure 2.--The total numbers of daughters born to ewes of different parity and retained in flocks A and B (during the years 1940-56), with those manifesting scrapie (figures within the blocks indicate percentages of total at risk).

period 1944-1961, of 1349 sets of twins recorded, 670 were of like sex (314 males and 356 females) and 679 were of unlike sex. We are assuming therefore, for the present purpose, that gestational twins are binovular. One-hundred and six sets of twins have been under observation until 4 1/2 years old in 6 flocks of low to high incidence--101 sets were both female, 4 a male and a female and the last were both males. In 17 sets both were affected, in 26 one was affected and one unaffected and in 63 both were unaffected. These data suggest that intra-uterine influences were not important and they were therefore examined in the light to the genetic hypothesis. The results are shown in figure 3, which relates only to the 83 sets of twins for which the necessary parental records were available. The number of sets in each category is small, but the results show a clear trend which is consistent with the genetic hypothesis.

Progenies of Different Sires in an Affected Flock

If scrapie is due to a recessive gene, then the attack-rate among the daughters of sires

Manifestation among 83 sets of fraternal twins from five medium- to high-incidence flocks

Category (table 2)	Mating ♂×♀	No. of sets of twins: Both affected	No. of sets of twins: 1 affected 1 unaffected	No. of sets of twins: Both unaffected	Total affected: obsd.	Total affected: *expd.*
Mixed progeny						
1	*ss*×*ss*	7*	2†	0	16	*16·1*
2	*Ss*×*ss*	5	2	0	12	*6·3*
3	*ss*×*Ss*	1	3	7	5	*9·9*
4	*ss*× 2·9 per cent. *ss*, 55·7 per cent. *Ss*, 41·4 per cent. *SS*	0	2	2	2	*2·2*
5	*Ss*×*Ss*	3	2	4	8	*4·0*
6	*Ss*× 2·9 per cent. *ss*, 55·7 per cent. *Ss*, 41·4 per cent. *SS*	1	5‡	16	7	*6·3*
Total	62	17	16	29	...	...
Uniform progeny						
7	*Ss*×*SS*	0	0	13	0	*0*
8	*SS*×*SS*	0	0	7	0	*0*
9	*ss*×*SS*	0	0	1	0	*0*
Total	21	0	0	21	...	...

* 1 pair of rams and 1 pair—1 ram and 1 ewe.
† 2 unaffected animals now 4½ years old.
‡ 1 pair—affected ram and unaffected ewe.

Figure 3

of different genotypes should be very different, provided that the proportion of dams of each genotype mated to each sire is similar. In only one flock has it been possible to assemble data relevant to this point. These are given in figure 4, from which it will be seen that the total attack-rate among 54 progeny of an affected ram is higher than among the 11 progeny of an unaffected ram, and 5 of an unclassified ram. When these attack-rates are subdivided according to the presumptive genotypes of the dams, a clearer picture emerges, and the 'closeness of fit' between observed and expected values is remarkably good.

Manifestation among the daughters born to three rams in 1955-56 and retained in the same flock (flock A)

Sire: Category	Sire: Presumptive genotype	Dam: Category	Dam: Presumptive genotype	Daughters: Unaffected	Daughters: Affected	Daughters: Attack-rate per cent. obsd.	Daughters: Attack-rate per cent. *expd.*	Flock attack-rate: Year	Flock attack-rate: Per cent.
Affected . .	*ss*	1	*ss*	0	11	100	*90*	1955	7·2
		3 and 5	*Ss*	23	17	42·5	*47·25*	1956	12·1
		7 and 8	*SS*	3	0	0	*0*	1957	16·7
Unaffected and unclassified .	*Ss*	1	*ss*	2	2	50	*47·25*	1958	12·6
		3 and 5	*Ss*	8	4	33·3	*24·8*	1959	18·6
		7 and 8	*SS*	...	...	...	...	1960	9·1

Figure 4

CONCLUSION

These results do not suggest that even in a very high incidence environment the manifestation is influenced significantly by environmental factors (special matings with ss and SS animals maintained in a similar high incidence environment have been made and the results are given in Oxford Paper 13), i.e. there is no good evidence that scrapie is disseminated through an infection of the usual communicable kind.

REFERENCES

M'Gowan, J. P. 1914. Investigations into the Disease of Sheep called Scrapie. Blackwood, Edinburgh.

Parry, H. B. 1962. Scrapie: A Transmissible and Hereditary Disease of Sheep. Heredity, 17, 75-105.

DISCUSSION

Dr. Dickinson:

You were saying that this has been published in Heredity? Those tables were the same? They have changed their shapes.

Mr. Parry:

They are the same and the material is known to you.

Dr. Dickinson:

One comment on that last table. It might clear up a misunderstanding from what I said this morning.

I said something about the applicability of these small samples to a large population understanding of scrapie. Now, this last table of yours was an instance of what I mean. What I am trying to say is that you have, when it comes down to the end of the issue, fairly small groupings of data. We have fairly small groupings of data and we both need to be in a position of saying about these small groupings--say that last table or the one before it or the ones that are to be presented by us--they are of a similar sort of size. Can you apply this, whatever it might be, to an understanding of scrapie as a scrapie problem. So here I say, you claim to have large records.

Mr. Parry:

This is only a pointer. We have a very much more substantial table.

Dr. Dickinson:

Can I just make this point. You are claiming to have very large records. This means you must have hundreds and hundreds of ram families and so you should be able to get a population assessment of what happens to progeny, but this would lead to a great understanding of the population, what happens on an average to the progeny of affected rams versus unaffected, simply a twofold classification, not a rarity. One should not talk of large populations to start off and then come down to 32 sheep. That is the trouble. This can be the data you selected out to prove your point.

Mr. Parry:

This is going to be dealt with by Mr. Draper in a minute in a much larger sample.

Dr. Gordon:

One point I would like to be clear about. In the course of your presentation, I think you showed two instances in which the progeny of two affected parents did not develop scrapie. This interests me greatly and may have a bearing on the evidence I presented yesterday about two susceptible parents sometimes producing resistant progeny. Of course, your hypothesis and mine differ in the sense that you believe the disease is exclusively transmitted hereditarily whereas I am thinking of two factors: A hereditary factor that determines susceptibility and an environmental factor presented by the transmissible agent.

I just wanted to be clear in my mind that this can happen; a ram that takes the disease and a ewe that takes the disease can produce a progeny that doesn't take the disease.

Mr. Parry:

It depends on how long you watch them, or they live.

Dr. Gordon:

How long have you watched these two?

Mr. Parry:

Four and one-half years. They are classified as I gave you this morning.

Dr. Gordon:

So they have lived $4\frac{1}{2}$ years without taking scrapie?

Mr. Parry:

Yes.

Dr. Gordon:

Small chance of taking scrapie after that.

Mr. Parry:

Yes.

Dr. Mulhern:

Mr. Parry, for the benefit of some members of our sheep industry who are attending this meeting, and who will later serve on an evaluation committee, it would be very beneficial to them for you to elaborate on the hypotheses of the recessive gene, so they have this background in weighing the over-all problem.

We spent all day yesterday showing the transmissible agent and that side of it. We would like to have the other side, from the genetic standpoint.

Mr. Parry:

Certainly. We proposed to do that in another paper.

Paper No. 12

NATURAL SCRAPIE IN SHEEP: ITS OCCURRENCE AND DISSEMINATION IN THE FIELD. III. THE EFFECT OF THE INTRODUCTION OF SCRAPIE AFFECTED SHEEP INTO FLOCKS AND PREMISES FREE OF THE DISEASE AND THE QUESTION OF RESIDUAL CONTAMINATION

H. B. Parry[1]

The effect of introducing scrapie affected sheep into flocks free of the disease is a problem of crucial importance in establishing whether contagion plays any significant role in the dissemination of scrapie under natural conditions.

The opportunity to observe over an adequate period flocks in which this situation has occurred are infrequent, and we know of no reliable reports in the literature.

In several flocks under strict supervision (B.S.S., Class III B.C.) the introduction of a ram which subsequently developed scrapie into a flock free of the disease has allowed certain pertinent observations to be made. These have been amplified by the deliberate introduction of females from a free flock into a badly affected flock, and by the setting up on our experimental farm of a contact experiment.

INTRODUCTION INTO A FLOCK FREE OF SCRAPIE OF RAMS SUBSEQUENTLY AFFECTED WHILE IN THE FLOCK

The Subsequent Occurrence of Scrapie in the Flock

(a) All sheep of the same breed and similar breeding lines.--The results are given in

[1] Nuffield Institute for Medical Research, University of Oxford, Oxford, England.

figure 1, for two flocks, each of which has had 2 of its stud rams go with scrapie since 1955. No cases of scrapie have occurred in any home-bred sheep in these flocks nor has any report of scrapie in sold sheep been reported.

The Occurrence of Scrapie in Females Mated by Rams Affected at or Shortly After the Time of Mating

The results are given in figure 2 for 3 rams in 2 flocks. 166 ewes were in-lamb to matings

Introduction and use of rams later affected with scrapie into a free flock

(Breed N° 72).

1	Year.	Total.	Age composition (± ½ yr.). 1	2	3	4	5	6	7	Cases of scrapie.
Flock ewes.	1915-51	100-200								0
	1952-59	200±50								0
	1960-64	mean 247·5	5·25	51·5	39·75	40	39·25	31·5	40·25	0
		%		55·2				44·8		
Stud rams affected	B.R.E. 59/10 Used 1959,60,61.									April-June,62.
	B.C. 60/24 Used 1960, lent 1962, returned June 1963.									June-Sept. 63.
	B.B. 61/134 Used 1961,62.									Suspect Jan. 64
2. Flock ewes.	1926-51	80								0
	1952-59	80-120								0
	1960-64	mean 128·6	0	31·4	20·6	16·2	16	15·8	26·6	0
		%		53·0				47·0		
Stud rams affected	K.A.C. 55/36 Used 1955,56,57									July,57 suspect. Jan. 58 definite.
	B.F.C. 58/5 Used 1958,59,60.									Sept. 60.

Figure 1.

It may be noted that in both flocks some 45 percent of the breeding ewes were over 4½ years old for the last 5 years.

(b) Affected Purebred Rams and Commercial Females.--In a flock of 700 ewes breeding some but not all of its flock replacements 2 rams used between 1955-57 died of scrapie in 1958. This flock has carried within it since 1959 a small flock of my 'black' sheep, all of which have developed scrapie there when they have reached the age of manifestation. Thus the environment of this flock remains suitable for the development of natural scrapie.

No case of scrapie has occurred in the commercial females or other rams.

by these rams 9-1 month prior to the onset of clinical signs, and 58 have been under observation at least 3 2/3 years thereafter.

No case of scrapie has occurred.

The Occurrence of Scrapie in the Daughters of an Affected Ram

Figure 3 gives the results for the years 1955-64 of such a situation in a clear flock of the same breed. Of the 46 daughters retained 33 have been under observation until 4½ years of age. No case of scrapie has been seen among these progeny and the flock has remained completely free (except for this

The occurence of scrapie in ewes in-lamb to rams developing clinical scrapie at or shortly after the mating (Breed N° 72), 1956-64.

Ram Identity	Ram Scrapie status	Ewes Age at mating	Total N°	N° under observation (years) ⅔	1⅔	2⅔	3⅔	4⅔	5⅔	6⅔	7⅔
KAC.	1956. 9 months before onset.	2½	41	4	5	18	11	0	0	1	2
		3½	15	2	2	2	7	2			
	1957. affected stage 1a	3½	3		1	2					
									Total		59
									Cases of scrapie		0
									Scrapie in flock		0
BFC.	1960. affected within 1 month.	2½	24	3	1	4	16				
		3½	11		1	1	9				
		4½	10		3	0	7				
		5½	9			3	6				
									Total		54
									Cases of scrapie		0
									Scrapie in flock		0
BZ.	1957. 9 months before onset	2½	12	4	2	3	2	1			
		3½	18	8	1	8					
		4½	12	7	3	2					
		5½	1	1							
									Total		53
		6½	10	4	1	5			Cases of scrapie		0
									Scrapie in flock		1

Figure 2

The occurrence of scrapie (1956-64) among the daughters of a ram introduced into a clear flock and subsequently affected there (Breed N° 72).

Ram introduced august, 1955, and affected, april 1958.

N° of daughters under observation until age (yrs.).

Born	1½	2½	3½	4½	5½	6½	7½
1956	15	15	12	7	4	1	1
1957	12	12	10	8	6	5	
1958	19	19	19	18	18		
Total.	46	46	41	33	28	6	1
Cases of scrapie.	0	0	0	0	0	0	0

		1920 - 1955.	1956-1960.	1961-1963.
General flock.	N° of ewes	120-200	220	130
	% older than 4½ yr.	(~30-40)	~40	48.2
	Cases of scrapie	0	0	1 (Nov 63)*

*Ewe SF 40-58/54 { SF9-54/00R o.k. to 5½ yrs. — SF9 affected at this time and produced many grey rams and black sheep.
SF40-54/153 o.k. to 5¼ yrs. { SF 26-47/00R by grey sire. / SF 40-51/180.

Figure 3

affected sire) since its foundation in 1920 until the fall of 1963, when a case occurred in a ewe SF 40-58/54 nearly 6 years old with a suspicious parental background.

In this flock nearly 50 percent of the ewes have for some years been older than 4 1/2 years of age.

Figure 6 gives the results in a situation with many cases in the affected flock over the past 10 years, in which 265 females of the free breed have been exposed, 34 for 4 years and 30 for 5 years.

No case of scrapie has occurred in either flock of the two free breeds.

Introduction of 'white' females of the same breed and closely related breeding into an affected flock.

Age in years at intro.^{n}	Number surviving 1959	60	61	62	63	64($^{1}/_{2}$yr).
$5^{1}/_{2}$	3	3	3	3	2	2
$4^{1}/_{2}$	1	1	1	1	0	0
$3^{1}/_{2}$	4	4	3	3	3	3
$2^{1}/_{2}$	2	2	2	2	2	1
$^{1}/_{2}$	4	4	4	3	3	3
Total at risk	14	14	13	12	10	9
Losses – scrapie	0	0	0	0	0	0
Losses – other	0	0	1	1	2	1
Scrapie cases in rest of flock.		20	30	37	15	2

Figure 4

INTRODUCTION OF FEMALES FREE OF SCRAPIE INTO AN AFFECTED FLOCK WITH A HIGH INCIDENCE

The results are given in figure 4.

It will be seen that 10 of 14 ewes have survived at least 4 years and 9 to 4 1/2 years without any sign of scrapie, which has been rampant in the rest of the flock.

INTERMINGLING ON THE SAME PREMISES OF A BREED AFFECTED WITH SCRAPIE AND OF A BREED FREE OF SCRAPIE

Figure 5 gives the results obtained in a situation with only occasional cases in the affected breed and of recent date.

CONTACT EXPERIMENT ON PREMISES CONTINUOUSLY CONTAMINATED FOR 6 YEARS

In 1957 the field evidence was pointing to the conclusion that natural scrapie was not contagious in the usual sense, but was mediated in some way through the hereditary mechanism.

An opportunity occurred to acquire some 35 ewe lambs from one flock by 5 sires, half out of affected dams and half out of unaffected dams. There were approximately equal numbers of progeny by each 5 sires, all of which were certainly greys and lived to over 4 1/2 years old. To these were added purchases from 3 other flocks of the same breed considered (as far as one could judge at that time) to be free of scrapie, and groups from two clear breeds

Effect on sheep of a clear (class SC1) breed of immediate contact with sheep of an affected (class SC3) breed with clinical cases.

		1958-59	60	61	62	63	64
Class SC1 breed (N° 76)	Flock Ewes Number	185	193	192	180	153	100
	Age	← approx. 45% over 4½ years of age →					
	Cases of scrapie	0	0	0	* 0	0	0
Class SC3 breed (N° 72)	Flock Ewes Number	72	82	126	150	180	217
	Cases of scrapie	0	0	0	0	2	0

* 10 yr-old ewe tested for presence of TS A — negative in sheep and mice (to 21 months).

Figure 5

Effect of intermingling flocks of two breeds, one affected (class SC3) and one clear (SC1), 1955-1964.

Class SC3 breed (N°72).	Year.	1955	56	57	58	59	60	61	62	63	64
	Cases of scrapie	18	11	11	8	21	29	19	9	17	

			N° of females and duration of contact in years				
		Total.	1	2	3	4	5
Class SC1 breed (N°79)	From conception.	127	44	23	25	16	19
	From age 1½ yrs	68	19	26	9	10	4
	4½ yrs	70	11	24	20	8	7
	Total.	265	74	73	54	34	30
	Cases of scrapie	0	0	0	0	0	0

Figure 6

(these breeds and the flocks of origin still remain free). All sheep were of the same age group and came under experiment when 1-1½ years of age.

The experimental plan and predictions made on 21 January 1958 are shown in Figure 7. Since that date these sheep have been run together as a single flock (except for short periods at mating, etc.) and have been in continuous contact with natural cases of scrapie (often as many as 10) and latterly in intermittent contact with cases of artificial

Sheep in experimental contact with natural scrapie by contiguous contact, coitus and contaminated premises, 1958-64.

I Plan and prediction, 21 Jan. 1958.

Group.	I	II	III	IV	V
Breed and flock.	N° 72 A. B.	N° 72 A.	N° 72 C. D. E.	N° 77.	N° 70.
N° of sheep.	17 2*	16	2 4 7	10	10
History of scrapie in flock of origin.	Over 10% in 1957.		None in past 5 years.	No cases known in these flocks or breeds.	
History of parents.	Dams affected. All sires 'greys' and approx equal N° of daughters in both groups.	Dams not affected.	No cases yet recognised: under supervision.		
Presumed genetic constitution.	All ss or Ss.	Ss or SS with some ss.	SS with some Ss and perhaps an ss.	SS (probably no Ss).	
Expected incidence of clinical scrapie by 1962.	50-75%.	20-30% but may be more.	<10%	0	0

*Both parents affected.

Figure 7

post-inoculation scrapie; they have been mated with natural 'black' rams subsequently affected and with rams affected at the time of mating. They have been bred from and treated like an ordinary farm flock. The farm must be considered as heavily contaminated since there are normally 10-15 cases about at any one time.

The results are shown in figure 8. It will be seen that scrapie has occurred in 3 groups

Sheep in experimental contact with natural scrapie by contiguous contact, coitus and contaminated premises, 1958-1964.

II Results to 21 Jan. 1964.

Group.	N° of ewes at start.	Deaths from		N° of survivors to age (yrs.)		
		Scrapie.	Other causes.	3½	4½	6
I	19	10 (53%).	6	5	3	3
II	16	7 (44%).	7	7	3	2
III	13	1 (7·7%).	9	5	4	3
IV	10	0	5	6	6	5
V	10	0	5	10	6	5

Figure 8

only (of an affected breed) and that no case has occurred in the free breeds. It is encouraging that the general predictions of the attack-rate of scrapie in each group made 6 years ago have proved to be so close to observed results. These predictions were based entirely on the recessive hypothesis and are thus strong evidence of its usefulness.

CONCLUSIONS

No evidence whatever can be found to support the thesis that natural scrapie is due to a communicable infectious agent spread by contiguous contact, coitus, contaminated surroundings or residual contamination of premises.

The recessive gene hypothesis has allowed predictions to be made of the subsequent scrapie attack-rate, which have proved remarkably accurate and useful in practice. This accuracy of these predictions based on the genetic hypothesis provides considerable support for the involvement of a hereditary genic component in natural scrapie.

DISCUSSION

Dr. Gordon:

I don't want to waste time but I think you referred to running an affected Suffolk flock with Dorset Downs and there was no evidence of spread from Suffolk to Dorset Down.

On the evidence I presented yesterday, this is not surprising. If the susceptibility of your sample of Dorset Downs was similar to those I have tested, (all of which were resistant,) then I would not expect scrapie to occur in these animals, and one cannot readily assess the possibility of spread at pasture unless sheep known to be susceptible are exposed by contact to the challenge of infection.

Mr. Parry:

To the artificial disease, Dr. Gordon, not spread by contact in the field.

Dr. Gordon:

It is almost impossible to get this kind of evidence experimentally until sheep of predictable susceptibility have been obtained. This is what we are attempting to produce; a population which is 100 percent susceptible and another which is resistant. With these two kinds of sheep it will be possible to determine how may genes control susceptibility and any number of experiments, including spread by contact, can then be undertaken. To suggest that there is no spread by contact because of an experiment, such as the one you have just described, is arguing on false premises because you don't know whether your Dorset Downs are susceptible or not.

If they are anything like my Dorset Down sample, they are not susceptible, so you won't get the disease spreading to these sheep in any case.

Mr. Parry:

I am not interested in spread of your artificial diseases, nor post inoculation diseases. I am only concerned whether natural scrapie tends to spread in the field.

The question of the artificial disease, I will talk to later, because I think our views on it are not going to be very dissimilar.

Dr. Gordon:

This will be interesting, because when you visited Compton on one occasion to obtain material from affected sheep for examination for myopathy, I showed you natural and experimentally-affected animals and, at that time, you could not distinguish which was which.

Mr. Parry:

No, I don't pretend to. This is not the point. The point is and, Mr. Chairman, I must reiterate this with the greatest firmness, that we will talk only of natural spontaneous scrapie at the moment. This may be quite different from the artificial post-inoculation scrapie. The relevance of data on the artificial disease to the question whether the natural disease is spread by contagion in the field, should not be prejudged, and I only wish to speak of what we have observed about the natural disease.

Dr. Dickinson:

You would agree that the only relevant information--and here I am taking up the kind of point that Dr. Gordon has been making--to this issue would be to expose sheep which could get scrapie--

Mr. Parry:

What kind of scrapie?

Dr. Dickinson:

I am not interested.

Mr. Parry:

This is the point. Do Dorset Downs get natural scrapie?

Dr. Dickinson:

I have heard of a case.

Mr. Parry:

Good, you have data which I haven't access to.

Dr. Dickinson:

This is the point in which I am sure the people here from the field are most interested. We will keep being asked, and I will be asked tomorrow, have you got evidence in sheep of contact transmission. And the point here is, you can only get evidence of contact transmission of natural scrapie if you want to go along with Mr. Parry here, or myself here, in saying induced scrapie may be different. You can only get evidence for contagious transmission of natural scrapie if somehow or other you can establish that one group of sheep are identical genetically with another large group of sheep, and hopefully some sheep in both groups can get natural scrapie and you can demonstrate by environmental manipulation that one group does not get the disease and the other group does get it, and that you can tie up the fact that the groups which get scrapies are the ones that are exposed and others are not exposed. But we rule ourselves out of court automatically so long as we say that we cannot even edge in on this problem.

I suggest we think in terms of natural scrapie, also induced scrapies, they are plural words, not singular. We can now say which agent it is induced with, and there are many different agents. These may be different. Suffolk scrapie may be different, naturally, from Cheviot scrapie. These two things may be different in these cases. But I would suggest that the point here is, at this stage, to decide what is evidence for what happens. You do start with the Cheviot agent--Dorset Downs did not go down.

We may someday find an agent to which they do go down, but we are waiting for those data. But useful evidence, at the moment, to suggest that they might in fact be the breed which are absolutely resistant, willy-nilly, to whatever agent they are challenged with, such evidence is lacking. This absolute resistance may in the end prove to be true for some breeds, but this is the only starting point for us to use. In fact, we can, say, take a breed which doesn't get scrapie, and which has lots of inducible scrapie, and see what happens there.

This is the point Dr. Gordon is making, and I am sure we will both agree that this doesn't necessarily say anything about natural scrapie, but this is the only point at this stage that we can make any attempt to answer.

Mr. Parry:

May I refer you to the experiment in which white Suffolk ewes were introduced into an infected environment?

Dr. Dickinson:

We must both rule our data out. You would say they didn't get it because they hadn't the gene, and I could equally well say, again without any supporting evidence, they were resistant, or you didn't know them long enough. We just get nowhere with these kinds of data.

Mr. Parry:

We are not saying whether it is due, you know, to an infection or the gene. All I am reporting is an observation.

Dr. Dickinson:

When you record your observation, what age is that one case that went down, $5\frac{1}{2}$ years?

Mr. Parry:

Yes.

Dr. Dickinson:

I would pinpoint this, because I will be coming back to this. Dr. Gordon spent his time yesterday morning saying that it is funny how these suggestive case of contagion crop up in old sheep. And I will be worrying with it tomorrow.

Dr. Stamp:

One point that is away from the present discussion. I didn't like the way in which

Mr. Parry concluded that because material from this one sheep we are talking about, when introduced into mice and the mice hadn't gone down, and when introduced into sheep and the sheep hadn't gone down, concludes that it wasn't a case of scrapie. This could not be further from the truth. Anyone who has worked with sheep material in mice will know full well that this does happen on first passage. Those who work with sheep material in sheep will also know full well that this often happens. This is no evidence as to whether the disease is scrapie or whether it is not scrapie unless it is followed through further passage.

Mr. Parry:

Dr. Stamp, this animal was used as a control against a natural case of scrapie. They were inoculated 21 months ago. All the cases in mice have gone down with scrapie between fifteen and eighteen months. None have gone in this normal one. My sheep, I have Swaledales, because I knew of Dr. Gordon's report of high incidence in them, and I couldn't get Herdwicks; none have gone down after 21 months. I don't understand this. I don't pretend to. I am hoping someone will help me.

Dr. Gordon:

It is very simple, because it just depends on the sample you happen to get. And this is the point I want to make quite clearly. If the sample that is exposed to infection is not susceptible, you get a negative result. But you are arguing from a false foundation, because if you don't know the susceptibility of your animals, and you get no spread of the disease the whole process is a waste of time because the exposed animals may be resistant.

Mr. Parry:

How did you know the susceptibility of your original Swaledales?

Dr. Gordon:

By testing.

Mr. Parry:

Exactly; like I have. You didn't know anything about the genetics.

Dr. Gordon:

I knew nothing about genetics.

Mr. Parry:

No more than I.

Dr. Gordon:

I only was anxious to know whether they were susceptible.

Mr. Parry:

Is this not circular?

Dr. Gordon:

No, not in relation to our tests, because parents breed in isolation and after they have produced the next generation the parents are removed and tested for susceptibility. The offspring can then be classified according to the susceptibility of their parents and the offspring of two susceptible parents are likely to be susceptible in a high proportion of cases.

Mr. Parry:

This raises a terribly difficult question. How do you assay transmissible agents? I do not pretend to know about that, but I hope to ask some questions or learn something in the course of the meeting.

Dr. Dickinson:

A couple of comments on this particular point: One is that we tried to get a variety of scrapie agents going from different sources. We failed to get a Dalesbred agent going in sheep. We have kept failing in this direction. We failed to get a Suffolk agent going in sheep within 2 years of challenge. We have kept repeating this one and we failed. This is funny, and so all that Dr. Stamp was trying to bring out here is that some people have been lucky, one or two people in this country, now Zlotnik, feeding brains to mice. You have been lucky in some of your cases and we have been lucky in some of our cases, but have always failed in some of our cases.

The only place I know that had 100 percent success so far in getting scrapie to go whenever they have started isolating it is in Iceland.

Mr. Parry:

The point is immaterial and purely an additional one to this particular point. I didn't want to raise it because I want some guidance on this question of assay. I find it desperately difficult. However, I will leave that.

Dr. Dickinson:

I am simply trying to say as things stand, as far as we see, you just can't assay for a natural agent because it involves using first-passage material and animals. It may fail, it may succeed, and that is it for the moment. We don't understand it in our experience.

Dr. Mulhern:

This one flock I think showed that there were by years 18, 11, 11, 8, 21, etc., scrapie cases per year -- one hundred and thirty-three cases in all. Was this in anyway artificial scrapie? Was this a normal number of cases in one particular flock?

Mr. Parry:

Yes, natural cases each year. The flock size varied from 150 to 250 sheep.

Dr. Mulhern:

The interesting point is that this is nearly as many cases in one flock as the total which have been found in the United States during the last 10 years.

Mr. Parry:

Yes. We are not bothered with this and still maintain the profitability of our sheep enterprises in spite of this.

Dr. Gordon:

Is this flock owner still in business?

Mr. Parry:

Yes, he has jolly good technical advice, Dr. Gordon.

Dr. Gordon:

Perhaps to accompany that he has a lot of money as well.

Mr. Parry:

No, he is a genuine working farmer.

The susceptible strains in contact I can't tell you, but we have another instance of Mashams in contact, who are the product of two breeds -- a cross between two breeds -- which almost certainly carry the trait. We have had one case, in I think 100 ewes which have been running in contact for 4 years, but I didn't present it because they are not individually eartagged and I cannot therefore, you know, vouch for it. But there doesn't seem to be any spread to them. I don't know whether you would count them as susceptible stock or not. I wouldn't know.

Dr. Wedman:

This incidence in Dorset Downs which we somehow neglected as not too important in the United States has been coming up. Undoubtedly you have some kind of veterinary records in the country as a whole, in the whole of Britain, too, is there no record of any Dorset Down coming down with scrapie?

Dr. Gordon:

Yes, there is, Dr. Stamp has told me of two he received at Moredum with natural scrapie. Now, I would like to make it quite clear that we have tested only a sample of Dorset Downs. We cannot test the whole breed. But I would imagine that if one injected the scrapie material into the whole breed of Dorset Downs, the incidence of infection, in my view, would probably be very much less than if we injected scrapie material into the whole of the Cheviot breed. Of course, one method of eradication in the United States that could be adopted would be to inject the scrapie transmissible agent into all the sheep in a flock where the disease appears. This would pick out all the susceptible animals and leave the resistants. But in addition to leaving the resistant sheep, you might leave a number of carrier sheep, and this would be a dangerous policy.

Dr. Goode:

Dr. Gordon, I am sure you mentioned this as a matter of academic interest and not as a suggestion.

Dr. Gordon:

I am merely introducing a humorous remark to bring a little relief to a rather tense situation.

Dr. Mikel:

Dr. Gordon, you mentioned yesterday that in spite of the fact you apparently have a high incidence of scrapie in sheep, and in spite of the fact that you were able to get a very high incidence of scrapie in goats when you gave them brain material from scrapie sheep,

nevertheless, you don't, as far as you know, have scrapie in goats in Britain. Does this suggest that there may be a difference between your scrapie which has been injected versus the scrapie that occurs naturally?

Dr. Gordon:

These are differences between the experimental disease in sheep and goats. Goats are highly susceptible to experimental infection whereas the susceptibility of sheep is unpredictable. There are also other differences and this is where the problem of finding an experimental method for studying the epidemiology lies. Goats are not suitable for this work; it must finally be done in sheep.

As to the question of whether scrapie occurs naturally in Britain in goats, it is probably rare, but Dr. Stamp can answer that better that I can.

Dr. Stamp:

I am going to give a paper on this tomorrow.

Dr. Mikel:

Is there any way or any thinking about what the percentage of susceptible sheep would be if you could do this sort of thing?

Mr. Parry:

Artificially susceptible?

Dr. Gordon:

In assembling groups of sheep from 24 different breeds for susceptible test, we usually obtain each breed from several different flocks and there was a wide range of variation in susceptibility. Sheep of the same breed from one source might have a number of susceptibles and from another source complete resistance. For example, the Suffolks that we tested were from three sources; two of the sources were kindly supplied by Mr. Parry and I asked him to select sheep from farms where there was no history of scrapie at all. The reason for no history of scrapie on these farms may very well be that there are very few susceptible sheep there, and these samples might be regarded as biased in favor of selecting resistances. The other group of Suffolks came from a particular flock in Cambridge. There was some question raised as to whether they were absolutely purebred Suffolks, but every case of scrapie that developed in Suffolks was in this group of sheep, and not in the other two groups. So that this just emphasizes how unpredictable the susceptibility of sheep can be. This is a point I was trying to make yesterday. It is almost impossible to carry out experiments in sheep of unpredictable susceptibility. That is why we are trying to produce populations of sheep classified for susceptibility or resistance. With animals of this kind it will be possible to undertake experiments that may throw more light on the epidemiology of the disease.

Mr. Parry:

The point about which, Dr. Gordon, you aren't fully aware, is that the Suffolks you had did, in fact, later develop scrapie in a small incidence in both flocks. However, that is beside the point. It just confuses the issue a little more.

The point is that as far as natural scrapie is concerned, we have been fortunate enough to assemble some data which may be submitted to statistical analysis. My colleague, Mr. Draper, has kindly done this for me and he will introduce the whole topic of what sort of ratios you require for the genetic hypothesis, particularly the recessive gene hypothesis, because of the data we have shown you, you will realize that it probably isn't dominant or sex-linked.

THE HEREDITARY MECHANISM IN THE CAUSATION OF NATURAL SCRAPIE IN SHEEP. I. THE DATA REQUIRED TO DISCLOSE HEREDITARY INFLUENCES IN ANIMAL DISEASE AND ITS BIOMETRICAL TREATMENT

G. J. Draper [1]

The present paper will be concerned with certain preliminary questions relevant to the general problem of examining various alternative hypotheses and to the quality and statistical treatment of the necessary data.

Partly because of the extremely variable age of manifestation of the diseases, together with the effects of culling, and deaths from other causes, it is extremely difficult to discriminate between different possible hypotheses concerning the mode of transmission of scrapie and, in particular, to examine hypothesis postulating a genetic mechanism.

The problem is to attempt to reconcile the conflicting evidence which has been put forward and to see whether any one simple hypothesis will account for all of it.

One of the striking discrepancies is that between the results of our matings of "affected" by "affected" animals and Dr. Gordon's results on "susceptible" by "susceptible". This seems interesting and important; at the moment it seems that the best one can do is to assume that there is some essential difference between the 'natural' disease and the 'artificial' one.

A greater difficulty is the contradictions in the data on the possibility of transmission by contact. We shall assume that for our data in our circumstances this does not occur, for we have no reason to think otherwise. If this assumption is accepted two views on previous reports are possible. First that these are based on inadequate observations or, secondly, that there is some essential difference between the field conditions in the different cases. Alternatively, it may be that in certain environments sheep are invariably exposed to an infective agent (for the effect of this see the next paper) while in others they may or may not meet it.

What we claim to have shown is that the recessive gene hypothesis fits our particular set of data. Now it may well be that other hypotheses are also consistent with the data, and I want to consider one or two of these when we come to our large table later on. Suffice it to say, for the present, that our data are inadequate to discriminate between certain subtly different hypotheses which might be put up. The recessivity hypothesis fits the data - but if one wished to test it against other rather similar hypotheses one would need more extensive data.

For purposes of discussion one may divide the methods of looking at the data into three types.

First, one may consider the possibility of rejecting a given explanation of the data on purely logical grounds. Thus, for the recessive gene hypothesis, if one has two animals which are known with virtual certainty to be clear of the postulated gene and these when mated continually produce affected progeny one has extremely strong evidence against the hypothesis. Similarly if two affected animals are mated and produce several progeny which survive for some years without manifesting the disease then again the hypothesis appears to be untenable.

Secondly one can look at the large-scale epidemiological phenomena. The trouble here is that there are so many unknowns in the situation, in particular it is rather difficult to avoid circular arguments. It is easy to get involved in these, and the complexity of the pedigrees and interbreeding between different generations and so on aggravates the problem.

The third possibility, and the one we shall be concerned with in the next paper, is that of

[1] Unit of Biometry, University of Oxford, Oxford, England.

examining rather closely pedigrees on which one has a good quantity of reliable information. Basically one is concerned here to use the theory to make predictions about the fate of individual animals using knowledge on their parents. These predictions can be checked against the observations on each animal. Similarly one can determine whether other possible theories would have made equally good or better predictions. We have done such an analysis on a comparatively small number of animals for which the necessary information is available.

One important question which arises is that of how, using such methods, one can examine the hypothesis that some sort of infective agent is involved. If one has a very startling sort of epidemic, and if not much scrapie has occurred before, then it may be said that it is impossible to explain this on genetic grounds.

This may well be true, but one would want to know how large the epidemic was and how much information was available on the pedigree before deciding. There is a paper (No. 16) following the one on the analysis of our data which shows that, under certain circumstances, it is possible to get something that looks like an epidemic but is in fact of genetic origin. There is reason to believe that such circumstances apply in the case of scrapie. This is mathematically trivial but it is interesting in this context.

Having discussed various ways of looking at the data it seems appropriate to make a few points about the quality of the data. Dr. Parry has said a good deal about this, and it does seem as though the data are reasonably good to say the least.

The points may be summarised as follows:

1. A recording system of high accuracy is required, providing, in particular, permanent identification of individuals, reliable pedigree data, knowledge of possible sources of bias, e.g. those due to culling.

2. One would like losses from competing risks to be as small as possible.

3. Adequate objective criteria for diagnosis should be laid down and adhered to.

In connection with this question of the quality of the data I wish to make one or two points about the effects of possible errors in observation, diagnosis or recording.

It is not sufficient to say that these may occur. We know that they may occur. What we want to know is: If there are errors, in what way is it going to affect the inferences? People are going to suggest that there are such errors, that diagnoses are wrong, perhaps that there are errors in recording. We want to know what sort of magnitude would be required in these for it to upset our conclusions.

My own feeling is, that since the data fit the theory rather well, one should be disinclined to believe in these errors, since it would be asking for a curious coincidence that a wrong theory should combine with other errors to produce a good fit. (Though this does not dispose of the objection that the whole matter of the analysis has been wrongly approached; it must be admitted that certain of the conclusions rest on a few critical matings and more evidence will be needed to confirm or disprove these conclusions.)

DISCUSSION

Dr. Dickinson:

It is a matter on which Mr. Draper and I have a mutual understanding, in the sense that I know what he is meaning when he says, "the circular argument," which he is going to avoid, but I have a shrewd suspicion that there are probably only three or four more people here who know what he is going to avoid.

Could you please amplify this circular genetic argument which appears very prevalently in Mr. Parry's Heredity paper. Can you justify that every single ram so far as I can understand it in that paper is classified on the basis of its progeny except affected rams?

Mr. Parry:

That is not true.

Dr. Dickinson:

That is what the words say.

Mr. Parry:

No.

Mr. Draper:

One is assuming that the gene is passed on in a certain way. One wants to classify certain

rams or certain ewes; one can look at their progeny and classify them.

One then goes back and says on the basis of that classification, you can predict what their offspring are going to be and then look at the predictions. This is what I am going to try to avoid doing. It is rather unfair to suggest this is what Mr. Parry did in that there is a great deal of information on these animals.

Dr. Dickinson:

I have Parry's Heredity table, but this is table 2 on which he bases much of his conclusions. I will read you the sire column.

Now if there is an avoidance of the circular argument, he will say about the sire, it has one unaffected parent and one affected parent. If you hear those words, you know there is avoidance of the circular argument.

I will read this column: Class 1 "affected"--There is no argument.

Class 2, unaffected with several progeny affected.

Class 3, affected.

Class 4, affected.

Class 5, unaffected with several progeny affected.

That same thing is a circular argument.

Classes 5, 6, 7, and 8, unaffected with no progeny affected, circular argument.

Class 9, affected.

Mr. Draper:

All right, what happens if you say "unaffected with one progeny affected"?

Dr. Dickinson:

You would be saying that.

Mr. Draper:

But how would you classify the ram?

Dr. Dickinson:

I don't try to, but you have to classify it as a heterozygote.

Mr. Draper:

You are using one small part of the information to classify the animal. The rest you can then use to confirm or reject your predictions.

Dr. Dickinson:

I would heartily agree. The bias is minimal so far as a large ram family is concerned; but so far as dams are concerned, it is utterly destructive.

Mr. Draper:

We are talking about sires.

Dr. Dickinson:

I was talking about group size, yes; but it seems to apply to quite a lot of dams as well.

Dams mean progeny group size is something like 2 and you put your feet right into the biggest pitfall that geneticists have ever fallen into the moment you start classifying a dam with progeny size of 2. I agree this bias minimizes when you have large family sizes.

Do you know family sizes for sires? There aren't very many animals in that table?

Mr. Draper:

For a family of size three, if one is used in classifying the parents two-thirds of the data can be used in checking predictions. Clearly one cannot classify accurately without other information on parents of dam, age to which it survived, etc. The probability of misclassification is dependent on such facts.

Having partly accounted for the sires, at any rate, Dr. Dickinson has turned to the dams. I think one ought to point out that the dams can be partly accounted for as well. I am not trying to justify the whole of this. A lot of the dams are classified on their parents. That is in the table, too.

Dr. Dickinson:

What proportion?

Mr. Draper:

Shall we go through it later, or bring it up tomorrow if you like?

Mr. Parry:

The purpose of the Heredity paper was merely to provide a prima facie case for further investigation.

Dr. Dickinson:

No.

Mr. Parry:

It says so. Never mind, this is our purpose. We have more data.

THE HEREDITARY MECHANISM IN THE CAUSATION OF NATURAL SCRAPIE IN SHEEP. III. THE RECESSIVE GENE HYPOTHESIS AND ITS QUANTITATIVE APPLICATION TO FIELD DATA AND STATISTICS

G. J. Draper[1]

In a paper in Nature (Draper and Parry, 1962) we analysed an earlier version of the material which I am about to discuss. This was a fairly full analysis and we looked at two or three other hypotheses in addition to that based on a recessive gene; there are of course yet others that we might have examined in the light of our data and one or two will be mentioned in the course of the present paper. The other main object of this paper will be to bring up to date some of the data presented in the Nature paper.

The data in figure 1 have been taken from one of the flocks which Dr. Parry has been studying and, arguably, applies only to this flock. How much one can extrapolate from this to other flocks and other breeds is open to question.

Before going on to discuss Table 1 and the various hypotheses which may explain it let us refer briefly to figure 1. These are the sort of data which have been dealt with by Dr. Parry. Two points emerge from this table.

1. That when "blacks" are mated against "blacks" the progeny are virtually certain to get scrapie.
2. That the one "white", SS, ram confers complete immunity from the disease.

This obviously lends support to a genetic explanation of some sort though the evidence is scarcely conclusive. It is of course completely consistent with the recessive gene hypothesis but equally it is consistent with the hypothesis of susceptibility due to a recessive gene. Again it could be explained in terms of a susceptibility or immunity

Manifestation of scrapie among progeny of certain matings.

	Progeny - age in years.							
	1	2	3	4	5	6	7	8
I. ss x ss (All blacks).								
N° at risk - total.	140	122	88	78	77	73	73	72
N° of affected - total.	0	2	45	68	71	71	71	72
N° expected - total.		5·0	52·0	67·5	72·1	71·3	71·9	71·5
II. ss x SS (All greys).								
N° at risk - total.	46	46	41	33	28	6	1	
N° of affected - total.	0	0	0	0	0	0	0	

Figure 1.

[1] Unit of Biometry, University of Oxford, Oxford, England.

Table 1.--Numbers of offspring from various matings affected with scrapie and those alive and well by given ages.[1]

Dam's presumptive genotype		ss				Ss				SS			
		No. alive at 9 months	Scrapie or surviving by			No. alive at 9 months	Scrapie or surviving by			No. alive at 9 months	Scrapie or surviving by		
			3 yrs.	4 yrs.	5 yrs.		3 yrs.	4 yrs.	5 yrs.		3 yrs.	4 yrs.	5 yrs.
Rams:													
Affected Rams	1	5	3,1	3,1	3,1	12	5,7	6,6	6,6	4	0,3	0,3	0,3
	2	20	15,2	17,0	17,0	28	6,16	10,10	10,9	4	0,4	0,4	0,4
	3	6	1,4	3,2	4,1	10	2,3	3,2	3,2	2	0,1	0,1	0,1
	4	15	12,3	15,0	15,0	3	0,3	2,1	2,1	1	0,1	0,1	0,1
Presumptive heterozygotes classified on data outside table	5	8	4,4	6,0	6,0	4	0,4	0,3	0,2	1	0,1	0,1	0,1
	6	3	1,1	1,1	1,1	2	0,2	0,2	0,2	--	--	--	--
Classified partly on present data	7	5	1,4	1,4	1,4	8	0,5	1,4	1,4	--	--	--	--
One unaffected ram with many unaffected progeny	8	22	0,19	0,17	0,16	14	0,12	0,10	0,8	1	0,1	0,1	--

[1] The first figure in each cell is the number of affected offspring and the second is the number still under observation and alive and well--at ages 3 years, 4 years and 5 years. The data at age 9 months are simply the number of animals alive at that age.

transmitted in some way by the sire, and so on.

One can attempt a rather more sophisticated analysis for the data in table 1. Such an analysis was described in the 1962 Nature paper.

In this paper we shall briefly indicate the way in which the recessive gene hypothesis can be tested using this table and then consider what light it can throw on other hypotheses.

The question of the way in which the animals were selected for this table and the way in which the parents were classified should be mentioned first.

The progeny in the table consist of all female animals born to sires in the years 1958-59 plus some male animals born of ssxss matings, plus all females born to the presumptive homozygous dominant ram in 1960.

As regards the classification of the animals; one of the presumptive heterozygote sires needs some information from the present table in order to classify him. In addition the presumptive homozygous dominant ram is classified on data within the table (but he has so many progeny that this can scarcely be thought to matter).

In order to test the recessive gene hypothesis one makes predictions about the proportions of affected animals from different types of matings and tests these against the observed data as given in the table. The problem is complicated by the varying age of manifestation and the fact that deaths are occurring from other causes. Thus in making the predictions one has to take into account the probability that an animal believed to be homozygous recessive will in fact actually manifest the disease by a given age.

This introduces another complication, and some further explanation of what we mean by the "manifestation level" and, in particular, why we have used definitions of two such manifestation levels.

In Parry's original Heredity paper a manifestation level was used which was conditional on eventual manifestation i.e. it was the proportion of observed affected animals which were affected by some given age - the manifestation level at that age. This cannot be used for making predictions but should be thought of as a descriptive statistic. In the Nature paper we were concerned to make predictions which could be tested against observations and for this it was necessary to introduce a differently defined manifestation level; this was

conditional on the animal being alive and well at nine months, i.e. it was the proportion of all homozygous recessives alive and well at that age which manifested the disease by some given age. Basically then, what one does is to estimate the manifestation level, make the predictions and check against the observations.

It will suffice here simply to indicate some of the ratios in the table; the more detailed analysis was described in the Nature paper.

Considering the data at 5 years of age we have the following proportion of affected animals given in the table at the foot of the page.

The overall percentage of affected animals from ss x Ss plus Ss x ss matings is 42% or 44% according as we include or exclude the third Ss sire. We would on the recessivity hypothesis expect half as many affected animals from Ss x ss as from ss x ss matings and a quarter as many from Ss x Ss matings.

These ratios do not seem very different from those observed. Similar calculations can be carried out at 3 years and 4 years. That at 3 years suggests rather a large number of affecteds from Ss rams by ss dams - but the numbers are very small.

We turn now to other possible hypotheses.

The following were considered in the Nature paper. [It should be emphasised that the results depend very heavily on the one SS sire, and to the extent that he is a very unusual animal may be misleading on this account--that is to say while the evidence is interpreted as suggesting rejection of the various hypotheses it may be that the true alternative explanation is that all the observed effects are due in some as yet undefined way to this one unusual sire.]

We considered and rejected--

1. The possibility of transmission depending simply on the ewe being affected.
2. That it depends on whether she is affected at the time of gestation and/or suckling.
3. That it depends simply on whether the ram is affected.

   We may also briefly consider a few other possibilities.
4. That transmission is genetically controlled by a recessive gene with incomplete penetrance.

It does not seem that the penetrance could be very small because we know that a large number of animals from 'affected' by 'affected' matings get the disease by age 4 1/2 years.

The possibility of a large penetrance factor, but less than unity, does not seem distinguishable from that of manifestation dependent on age--for there seems no way of determining whether animals which die from other causes would in fact have remained free of the disease (incomplete penetrance) or merely were destined to be late manifesters (variable age of manifestation).

5. One might consider next the possibility of a dominant gene with incomplete dominance, i.e. of some heterozygotes (which should be phenotypically indistinguishable from homozygous dominants on a hypothesis of complete dominance) failing to manifest.

If only a small number of heterozygotes fail to manifest most progeny with at least one affected parent should manifest. But it may be difficult to distinguish this from the recessivity hypothesis--at any rate if there is a large number of heterozygotes which fail to manifest.

Finally we turn to the problem of whether the hypothesis of a genetically controlled (by a recessive gene) susceptibility or of contact transmission is compatible with our data.

Presumptive mating			Alive at 9 months	Affected	% Affected
sire	dam				
ss	ss		46	39	85%
ss	Ss		53	21	40%
Ss	ss	including	16	8	50%
Ss	Ss	3rd Ss sire	14	1	7%
Ss	ss	excluding	11	7	64%
Ss	Ss	3rd Ss sire	6	0	0

The former possibility seems, with our data, indistinguishable from the straightforward recessive gene hypothesis. For if one has an environment such that all animals meet the infective agent and all recessives are completely susceptible this is logically equivalent to simple genetic transmission. If there were instead a high or low probability of infection we are then in a situation equivalent to that of incomplete penetrance. If animals of similar breeding become scrapie in one environment but not in another, or only when moved from one to the other, one then has a situation in which either susceptibility or simple contact transmission seems admissible.

When circumstances such as these are envisaged it seems possible that many genetic explanations or none might be considered.

The problem of distinguishing between contact transmission and some form of parental influence will usually or always be complicated by the fact that closely related animals will often have had the same environmental experience.

REFERENCE

Draper, G. J. & Parry, H. B. (1962). Scrapie in sheep: The hereditary component in a high incidence environment. Nature 195, 670-672.

DISCUSSION

Dr. Carter:

May I ask the diagnosis of presumed parent's genotypes on the small s progeny of both parents affected?

Mr. Draper:

Some of them are affected themselves.

Dr. Carter:

Either they were affected themselves or their progeny?

Mr. Draper:

Yes. These were classified on various criteria outside the table. For some that would be true and others would be classified on other progeny which don't appear in the table. Incidentally, some of these genotypes may be wrong, but I don't see that this affects the sort of arguments we are making.

Dr. Carter:

The heterozygote identified on the basis of progeny, those progeny used to identify them were not included?

Mr. Draper:

Exactly. As I said earlier, there is in fact one exception to this. It is one of the heterozygote sires. But I don't see it affects any of the inferences we want to draw.

Mr. Parry:

Dr. Carter, some of the heterozygotes would have been classified on one parent affected and themselves living beyond 4-1/2.

Dr. Dickinson:

Could we have your big table back, please? I would like to just comment on this, because it represents a difference with our own data.

On the last line down, this one ram which is labeled as big-S big-S, that means to my mind that it was unaffected, and you have interpreted beyond that. Here we have a situation that relates to 37 progeny. Is that it?

Mr. Draper:

Yes.

Dr. Dickinson:

Here we have a situation where 37 progeny from affected mothers have not gone down by a fairish number of years old. This is in sharp contrast with our own data.

This point I would like to bring out, therefore: It is my interpretation of this situation that this ram differs from many rams present in the breed, the several rams we have tested, the other rams you have presented data on yourself; so if you want me to frame my remarks into a question, I would ask you:

How many other rams themselves unaffected have been mated throughout all of your data, have been mated with affected

females?--that is to contrast this ram with a goodly number of others? I would like to know this rough proportion. But I want to bring out this point: so far as I am concerned, this particular ram here is different and is important. This ram may well have big contributions to make to curing scrapie as a problem, and as far as I see it this sticks out quite differently from all of the rest of the data you have presented.

Mr. Draper:

I entirely agree.

This animal, as I said, is in fact rather odd. We haven't got another animal we can definitely classify in that way. What is the interpretation of this, I don't know.

One interesting thing about him is that one of his grandchildren has gone down with scrapie, which is in fact consistent with the genetic hypothesis. One of these so-called gray progeny was mated back to an affected ram and produced an affected offspring.

And there are a lot more animals of this type coming along. But they are not classified.

Dr. Dickinson:

Can we have a rough answer to how many other rams?

Mr. Parry:

Twelve that we have had enough data completed; and there are, I think, about fifteen others in the pipeline.

Dr. Dickinson:

I am asking this question because we have one large flock in which 21 rams were used during the course of something like ten years. Twenty-one rams had left at least four offspring to a reasonable age. And of these 21, 18 had left affected progeny, even when you were down to observing only four or five or maybe six recorded progeny. Two more only had four progeny so the tests on them are inefficient. And one ram--this seemed to be a different one--out of 21, just one, had eleven progeny, not by affected mothers; just total progeny. It may well have been a number of those had affected mothers. I am trying to bring out the situation here that the kinds of rams which don't leave some affected progeny may very well be different from this one down at the bottom of yours. But they are rare, even when you don't take the most informative class of how many of them have been mated with affected ewes.

Mr. Parry:

The ewes in your flock, do you know their pedigree?

Dr. Dickinson:

I am simply citing the situation that this was the background of the kind of thoughts that were in my mind behind asking you how many rams you contrasted with this one. And you are saying something like 12.

Mr. Parry:

This was, I think, 11. We have had two others since. And the others aren't far enough advanced. It depends very much on the background of the private flock you are thinking of, I suspect. One can get this sort of situation.

Dr. Dickinson:

This just puts that flock into context. This was a flock that, shall we say, hardly had any scrapie up until 1952 or 1954, tried culling just in affected lines against the other one or two that there were, and he has gone on from grays to grays, strength to strength, and he now has a lot of scrapie and is now a worried man.

But the point here is, so far as you are concerned, he was starting in a situation which was free, starting with a large flock, bringing rams in. And whenever he brought rams in, bing! bing! bing!-- at least one of their progeny went down with scrapie; and this is funny, you must admit.

Mr. Parry:

This happens, and it depends on the flocks from which he has been buying his rams.

Dr. Dickinson:

You see, he is not chucking out his females and there is a sufficient rate to account for the fact right at the start, when he had his first scrapie case, did he have this line of rams which always were leaving an affected animal. The point I am trying to make here is that it doesn't fit at all easily, as a population in these sorts of terms, it makes you

think that rams which do not have affected progenies are rare and you are in fact saying that this ram is a rather rare ram in your experience.

Mr. Parry:

They are rare amongst the best flocks. I presume that you are dealing with a good flock. Actually the parents of the white ram on that table were both greys.

Mr. Draper:

I am not quite clear about your rams. We know a certain amount about ours; and as you know, Mr. Parry has suggested that in fact there is a selection in favor of these animals (Ss or SS) for breeding purposes. Where one is carrying out an experimental program presumably this doesn't occur. But if these rams have just been brought in, they are maybe being bought for good points from the point of view of breeding and bringing in scrapie at the same time.

Dr. Hotchin:

Are we discussing the genetics of the agents causing scrapie? It is my understanding there is pretty good evidence to show that this is a virus. Or, are we discussing the genetics of inherited susceptibility for which there seems a good case in terms of different types of sheep? Or, are we discussing the converse of that, which is a factor which endorses great resistance.

There seems to be quite an anology with the virus I have been working with some time in meningitis, which was first found in Mount Cullens where it was carried apparently in a genetic way and was inherited and transmitted out of several generations and there were various possibilities but all these now, over the years, have boiled down pretty definitely to these two things: The question of susceptibility of the host to a transmitted virus. I get the impression that we are trying to force genetic concepts onto a set of data where it really doesn't fit.

Just one more point. If you have a virus, it would be well to take into consideration the other facts of modern biology that would seem to indicate that where you have one, you probably have a family, you may have several substrains that cause these different symptomologies and you may have other viruses of a similar type of behavior which cause perhaps muscular lesions rather than neurological.

I would suggest that it would be useful to concentrate on one clear cutting and define it, and perhaps have some subsidiary definition that would take care of these rather vaguer ones.

Dr. Gordon:

I was interested in Dr. Hotchin's reference to inherited susceptibility to meningitis now known to be caused by a virus which at one time was thought to be a heriditarily transmitted disease. I think we have reached a similar cross roads regarding the cause of scrapie and to give expression symbolically to my tentative view I would replace the symbols SS,ss, in Mr. Parry's blackboard presentation by Rs Rs = resistance to scrapie and rs rs = susceptibility to scrapie.

It is suggested that scrapie is a virus. I still maintain an open mind and say it is a filterable transmissible agent. Someone will finally confirm whether it is or is not a virus.

I believe it possible that the genetic constitution of the individual determines susceptibility to scrapie as in the example that Dr. Hotchin has described, my third paper contains evidence which attempts to test the hypothesis that a simple autosomal recessive gene determines susceptibility. While it was found that two susceptible parents produce susceptible offspring in a large proportion of cases there was also a number of resistant exceptions. An explanation for these is where the problem rests at the moment. However, the work we have in progress ought to settle this question and also if one or more genes determine susceptibility. If more than one or two, I will be happy to leave the problem in the hands of geneticists to unravel.

Mr. Draper:

I think I will just make these points and leave it at that.

Point one. As we see it, there are two possible hypotheses.

If we can just confine it for the moment to two hypotheses and suppose that either the recessive genotypes in some way, in which I

as a nonbiologist don't pretend to understand, produce this transmissible agent, that is one hypothesis. The other is that the recessives are merely susceptible and that this infection, or whatever it is, is around a lot and the recessives get it. I don't see any way to distinguish between the two.

The other point that Dr. Gordon made: As I mentioned earlier, there is in fact this great difference in what you observed and what we observed on the natural disease, namely, that nearly all the ones that we predict will go down do so. The ones that you predict don't. This at any rate suggests there might be something even if it has to be modified.

Dr. Gordon has just said that there may be a multiplicity of genes involved. I think if there are more than one, on the data we have, you can't do much about that yet.

Dr. Dickinson:

Can I just take up this point raised by Dr. Hotchin? I think others might be in this same difficulty of trying to find what it is that Parry and Draper have suggested and have published data to support and how it is that I, tomorrow, am going to differ so radically from them. It is on a small point, but it is on a vitally important point. Parry and Draper are not dealing with any question of resistance or susceptibility unless we have a ubiquitous agent which every susceptible must meet. The hypothesis they are advancing is that whenever you have a susceptible animal, it has willy-nilly got to go down, and any animals which does not go down is not a susceptible animal. Tomorrow I will say and produce data from Suffolks to show that when talking in terms of natural scrapie in normal breeding and in experimental breeding work, you must talk not in those terms, but in terms of at least susceptibility and resistance to a pathogen which the sheep may or may not encounter.

There is a radical difference at issue whether there is a pathogen here or not. The notions to which we are opposed, as far as I am concerned, are meaningless words to try and wriggle out of the fact that there is a massive evidence to say that scrapie looks mightly like a virus and I couldn't agree more with the Doctor.

Mr. Timm:

In your big-S big-S ram, I gather that it was from a dam and a ram that were both grey?

Mr. Draper:

Yes.

Mr. Timm:

How did you determine that from its one progeny? I understood your last ram which was big-S big-S--

Mr. Parry:

Big one, was white.

Mr. Timm:

I understood you to say that came from a dam that was grey and a ram that was grey.

Mr. Parry:

Yes, both of them had affected progeny in the flock of origin.

Mr. Timm:

How did you determine that this ram was white then?

Mr. Parry:

By the test mating.

Mr. Timm:

Then explain it to me, I didn't understand it the first time. I am sorry, but I would like to know.

Mr. Draper:

It is not only a question of its being from two heterozygotes, it has lived six years without going down. This doesn't make it certain but it suggests very strongly that it is that. The suggestion is even stronger when some of its progeny are taken into account.

Mr. Timm:

You classify on basis of progeny.

Mr. Draper:

Classified on parents and age to which it survived, which is a ripe old age, and in fact, again you see this is the same point Dr. Dickinson raises, you have 37 animals there. You could use one or two to classify. The rest test the data.

THE HEREDITARY MECHANISM IN THE CAUSATION OF NATURAL SCRAPIE IN SHEEP. IV. THE RECESSIVE GENE AND APPARENT EPIDEMICS OF CONTAGION

G. J. Draper[1]

This paper is a matter of simple mathematics and should not therefore be at all controversial. What we propose to show is that it is possible to get something which looks like an epidemic when in fact the disease considered is of genetic origin. The question of whether this is what is happening in scrapie is another matter.

All I have done in this paper which is set out in full in Heredity 1962 is to set up an extremely simple mathematical model which demonstrates the possibility of the occurrence described above.

The conditions postulated are as follows:

1. The disease concerned is due to a single autosomal recessive gene.

2. It manifests after the age at which reproduction occurs.

3. There is selection in favour of animals carrying the gene.

Point (1) has been and will be the subject of much discussion and will not be dealt with here. Point (2) is incontrovertible. On point (3) we suggest that there is in the case of scrapie selective pressure, for breeding purposes, in favour of animals homozygous or heterozygous for the postulated gene (this selection pressure arising through the association of superior development and breeding features with these genotypes). The validity of this association has been questioned; further research may clarify the issue.

Having outlined the conditions, we shall give only a brief indication of the demonstration that they can lead to apparent epidemics, referring to the paper already mentioned for a fuller development of the argument. It should be emphasized in passing that no attempt is here being made to prove that scrapie is due to a recessive gene, or to fit any particular set of observations to the theory.

In order to show that certain observations on the spread of the disease can be explained in genetic terms we considered two hypothetical populations of breeding ewes with a given age structure and, initially, complete absence of the scrapie gene. It is a simple matter to calculate the expected gene frequency and expected numbers of "affected" animals in the progeny in successive years if sires of known genotype are used and the female progeny retained for breeding. With this set-up one may carry out the necessary calculations assuming that, over a period of years, sires of known genotypes are used, and it can be demonstrated that the proportion of affected animals can rise quite steeply. Figure 1 shows the sort of thing that can happen. This shows for two hypothetical flocks

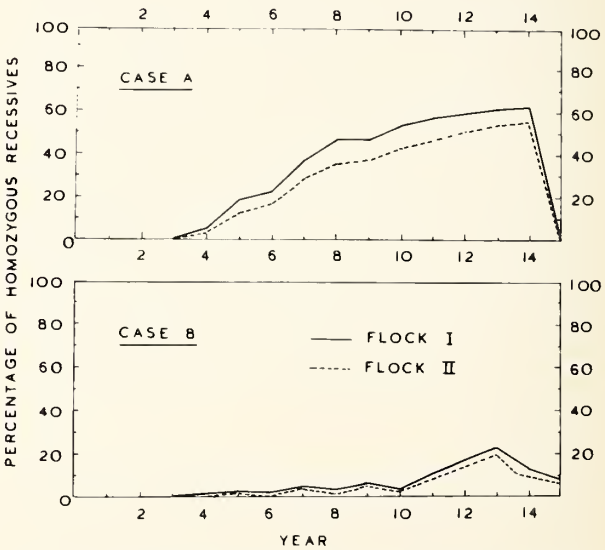


Figure 1.--Percentage of homozygous recessive progeny in successsive birth groups for the four hypothetical situations.

[1]Unit of Biometry, University of Oxford, Oxford, England.

of differing age structure what can happen if animals carrying the gene are used for breeding purposes. The top one is perhaps rather exaggerated, but not impossible in certain situations according to Dr. Parry's evidence.

It should be emphasised that the main factor leading to this phenomenon is that animals homozygous for the gene will very often not manifest the disease until after they and their relatives have been used, conceivably quite extensively, for breeding purposes.

A few points of explanation are necessary concerning the computations.

First, the percentage incidence is given for successive birth-groups, a birth group consisting of the animals born in any one year. Thus, since potentially affected animals will manifest the disease at various ages (and in a proportion of cases die from other causes before doing so) it is necessary to carry out further computations, using age-specific incidence figures to get the predicted number of cases during any particular year.

Secondly, there will in practice be certain unpredictable elements and random fluctuations not taken into account here. These will not, however, upset the conclusion which is simply that a certain phenomenon can occur. Other chance occurrences will simply modify it in particular cases.

Thirdly, no account has been taken of the culling of diseased animals or suspected carriers. This would modify the quantitative predictions of the theory, perhaps considerably.

An important practical point is that, as demonstrated in the lower line on the graph, one can have a low incidence going on for some time and rather quickly becoming much larger. This could account for sudden unheralded 'epidemics' since the low incidence might be missed or even concealed.

To summarise, we have shown that under circumstances which may be operative in the case of scrapie apparent epidemics may occur from the operation of a genetic mechanism.

REFERENCE

Draper, G. J. (1963) "Epidemics" caused by a late-manifesting gene. Application to scrapie. Heredity 18, 165-171.

DISCUSSION

Dr. Carter:

As I understand you, your assumption is that these rams are recessive homozygotes?

Mr. Draper:

Some heterozygotes. And one makes the assumption about what are being used in each year. In fact, you only need to make assumptions about the gene frequency and then go on and produce the gene frequency in the progeny.

Dr. Carter:

No selection for or against?

Mr. Draper:

It is suggested, the selection element occurs because the gene frequency builds up as a result of your selecting in their favor.

Dr. Dickinson:

Would it be fair to summarize your remarks by simply saying that, if you have a gene for anything, and select for or against it, allowing for drift, then you will change the frequency of that gene?

Though not using these words, this conclusion would have been pretty obvious to Gregor Mendel or have I missed something?

Mr. Draper:

You have missed nothing at all. I did say this earlier and I regard this as being entirely trivial. It was just interesting in this context.

CHRONIC INFECTION OF MAMMALIAN CELLS WITH VEE VIRUS

Henry J. Hearn, Jr. [1]

Venezuelan equine encephalomyelitis (VEE) virus is highly virulent in common laboratory animals producing febrile responses, high levels of viremia, paralysis in some species, and death within several days, generally irrespective of the route of administration (1, 2). A notable exception to its lethal effect is the rhesus monkey in which the disease is not necessarily fatal (2, 3). This agent is transmissible to humans usually causing a mild disease. In tissue cultures, a wide variety of mammalian and avian cells have been readily infected with VEE virus attended, in most cases, by a widespread lysis of virtually all of the cells of the culture (4).

During experiments in which L cells were tested for their susceptibility to VEE virus, rare "straggler" or normal appearing cells, almost obscured by cellular debris, were observed adhering to the glass surface of the culture flask. After several weeks, the entire sheet became repopulated from these few cells. Tests performed with the cell fluids obtained from these cultures disclosed small quantities of virus amounting to between 10^2 and 10^4 mouse lethal doses per ml of cell fluid. This contrasted sharply with titers of 10^8 to 10^9 mouse lethal doses of virus that were initially obtained 24 to 48 hours after inoculation of the culture. The chronically infected cultures could be kept in a viable state until terminated either by design or by contamination (1).

It should be mentioned here that in 1952, Bang and Gey (5) described a similar phenomenon with rat fibroblasts infected with eastern equine encephalomyelitis virus and in 1957, at the time when the chronically infected cultures were being investigated in our laboratory, Chambers (6) described another similar phenomenon with western equine encephalomyelitis virus. The mechanism underlying the chronic infection with eastern equine virus was reported to be the result of the outgrowth of resistant cells (7) whereas an interference phenomenon was reported as responsible for this effect with the western equine virus. In view of the evidence that supported these hypotheses, it was expected that similar mechanisms were operating in the cultures chronically infected with VEE virus. Cells surviving the lytic effect of the VEE virus infection appeared to possess certain morphological features that were different from the parent cell population, and the resultant chronically infected cell progeny appeared morphologically more homogeneous than cells of the original monolayer prior to infection. This plus the fact that the new cells were totally resistant to superinfection with homologous virus suggested the emergence of a resistant cell population. In addition, in view of the fact that VEE virus is thermally inactivated at the rate of approximately 1 log in titer every 18 hours at 37° C, fully or at least partially inactive particles were present in these cultures. These particles were very likely capable of interfering with the growth of fully mature, active virus. As our studies progressed, however, other factors had come to our attention. These will be presented as they relate first, to the virus and second, to the cells of the chronically infected culture.

First, the virus that was isolated from the chronically infected L cells demonstrated dramatic changes in its behavior in vitro and in vivo. Contrary to its relative quiescence in the culture from which it was derived, the virus proved to be extremely lytic, when given opportunity for growth by subculture in fresh normal L cells, more so in fact than the parent

[1] Virus and Rickettsiae Division, United States Army Biological Laboratories, Fort Detrick, Frederick, Maryland.

VEE virus (1). The variant strain of virus had, however, lost a great deal of its virulence in vivo as shown by the fact that it was not possible to produce a lethal illness in mice, guinea pigs, and rabbits when injected by peripheral routes (3, 8). This virus was, however, highly immunogenic and was used in animals as a live vaccine in a number of studies (9) some of which will be mentioned later. In addition, the variant virus produced very small plaques on chick fibroblasts and L cells under agar unlike the parent which produced large plaques under similar conditions (10). The agar was found to contain a component that was later found to inhibit the growth of the attenuated variant virus strain but not the virulent parent strain. More recent studies have shown that as the chronically infected culture continued its activities to periods up to and beyond a year, virus that had become completely avirulent for mice appeared. Such virus, however, was still highly infective for normal L cells.

Secondly, the chronically infected cells themselves exhibited new dimensions to their activities. Certain morphologic changes that had ensued have already been mentioned. Other, biochemical changes appeared. The cells demonstrated a markedly increased rate of acid production. While this phenomenon may have contributed to the inactivation and subsequent production of "interfering" virus mentioned earlier it was almost certainly indicative of a heightened cellular metabolic activity. In fact, if fresh medium was not placed on the chronically infected cultures with regularity especially when the cell sheets became confluent, a spontaneous generalized cell lysis occurred in which case it was necessary for the cell repopulation to begin again. Furthermore, we were able to demonstrate that a glutamine deficiency in the growth medium, ultraviolet irradiation of the cells (11) or simply transferring them from 1 flask to another similarly produced a spontaneous lysis, in addition to resulting in a 100- to 1000-fold increase in the production of virus. Attempts to preserve the cultures by freezing were met with extreme difficulty and no viable cells could be recovered even after short periods at the low temperatures. Quite obviously, the response of the culture to a variety of stresses was singularly uniform, namely, the destruction of most of the cells and a concomitant stimulation of virus growth. Studies to determine the number of infectious centers within the culture had revealed, however, that without the conditioning stress only one cell in approximately 10^4 could be shown to contain mature, infective virus at any one time. This was confirmed by fluorescent microscopy techniques with the assistance of Dr. John White from the Pathology Division at Fort Detrick. And, while it was sometimes difficult to prevent spontaneous lysis by transferring even enormous quantities of cells simultaneously, this could be carried out without spontaneous lysis when transfers were made if no more than 10,000 cells were employed. Cultures that grew after those cell transfers of low cell count appeared to be normal and were susceptible to reinfection with virus. These data, therefore, support the previously described evidence that 1 cell among approximately 10^4 within a chronically infected culture contained infectious VEE virus; the others apparently lacked mature virus. Whether the majority of the cells can be considered as normal, however, is questionable since they remained completely resistant in an environment containing active viral particles. Once removed from the infected environment, their return to a state of susceptibility suggested that they were not completely unlike normal cells and that their resistance to viral infection was of a transient nature.

In a recent review article, Wagner (12) has assembled evidence suggesting that chronic viral infections may be established and perpetuated in vitro by the cyclic production of virus and interferon. We have shown VEE infected cells to be producers of interferon. On the one hand, the maintenance of cells chronically infected with VEE virus seems feasible; on the other hand, it is difficult to explain the sudden breakdown in interferon's effectiveness that appears common to the diversity of stress situations mentioned earlier.

Cultures grown in the presence of specific VEE antiserum for several weeks thrived and apparently became dependent upon it for their well being. Upon removal of the antiserum, the culture lysed and increased amounts of virus were produced. This could be, of course, the result of the emergence of a new, susceptible cell population that was protected from virus

emitted spontaneously by the infected cells. Upon withdrawal of the antiserum, the cells without their protective shield were readily infected and lysed. It is interesting to note, however, that prolonged treatment with antiserum did not rid the culture of virus. Apparently the virus is capable of perpetuating itself intracellularly, perhaps through cell division. In other experiments, the cells of chronically infected cultures transferred in the presence of antiserum failed to show the spontaneous lysis that occurred in the absence of the antiserum. Antiserum, however, did not seem to prevent lysis of the cells after an ultra-violet irradiation and it appeared to be of limited benefit to the cells when they were cultivated in a glutamine deficient medium. Obviously, the role of antibody in the complex relationship between virus and cells in the chronically infected state is not clearly at hand.

Persistent in vivo host-virus systems present an infinitely more complex picture for study than the chronic infections in vitro. Perhaps a final few words in consideration of persistent infections in vivo with VEE virus, however, might be in order. It must be made clear at the outset, that evidence for latent infections of animals with VEE virus is highly circumstantial. Certain occurrences, however, that gave some substance to this hypothesis have been observed over a period of several years. These will now be mentioned briefly. 1 The possibility of persistent in vivo VEE infections was initiated upon the histopathologic examination of 2 healthy appearing monkeys that had been administered VEE virus 6 to 7 weeks prior to their autopsy. Examination of brain sections disclosed some localized perivascular cuffing and other histological findings that resembled those usually found shortly after VEE virus infections. Not expecting this, no attempt to recover virus was carried out. 2 Following the injection of mice with the nonlethal, attenuated strain of VEE virus mentioned earlier, it was possible to trace its disappearance from spleen, liver, and brain tissues. This seemed chronologically related to the appearance of neutralizing antibody. Just prior to the loss of virus in the brain, however, a form of virus that was no longer virulent nor immunogenic for mice was encountered. It was detected solely by its ability to infect and destroy L cell cultures (8). Whether we had witnessed a genuine disappearance of the virus from the host or merely a step in its transformation into an undetectable state within the cell is not known. 3 Mice that were immunized with attenuated VEE virus resisted low levels of the heterologous eastern equine virun within 18-24 hours postvaccination. An interference phenomenon was implicated as playing a predominant role in the early stages of resistance but as the immune state to homologous virus increased, resistance to the heterologous eastern equine virus remained at the same level. Antibody therefore did not appear to be related to the heterologous immunity (9) in view of this, a resistance factor at the cellular level may have been present. The crucial question is whether this is the result of a unique cell-virus relationship resulting from the invasion, retention and ultimate integration of the attenuated virus into the cell's metabolic processes. From this point it is tempting to postulate further that certain events in vivo may be analogous to those that can be readily demonstrated in chronically infected cells in vitro. These events may be associated, among other things, with the resistance of the cell against subsequent viral invaders. The induction of increased viral growth by stress factors in vivo would be difficult to demonstrate. Virus produced in this manner would presumably be overcome by circulating antibody thereby precluding most of the detectable effects that accompany a fully developed infection.

Continuing further within the realm of speculation, one final point might be mentioned in closing. I have described many of the events that occurred as Venezuelan equine encephalomyelitis virus changed from a highly virulent cell-destructive infectious strain into one that is relatively benign. This has apparently been brought about as a sensitive equilibrium between viral and cell activities was achieved. In considering the infectious process associated with scrapie, it would appear that the development of this disease might in some manner represent a reversal of the sequence of events described for VEE virus. The validity of such a hypothesis, of course, must await further investigations.

LITERATURE CITED

1. Hearn, H. J., Jr., Brown, A. and Hardy, F. M. (1961). Alteration of virulence of Venezuelan equine encephalomyelitis virus after passage in L cell cultures and in mice. J. Inf. Dis., 108, 237-242.
2. Hearn, H. J., Jr. (1960). A variant of Venezuelan equine encephalomyelitis virus attenuated for mice and monkeys. J. Immunol., 84, 626-629.
3. Berge, T. O., Banks, I. S. and Tigertt, W. D. (1961). Attenuation of Venezuelan equine encephalomyelitis virus by in vitro cultivation in guinea pig heart cells. Am. J. Hyg., 73, 209-218.
4. Hardy, F. M. (1959). The growth of Venezuelan equine encephalomyelitis virus in various tissue cultures. Am. J. Hyg., 70, 21-27.
5. Bang, F. B. and Gey, G. O. (1952). Comparative susceptibility of cultured cell strains to the virus of eastern equine encephalomyelitis. Bull. Johns Hopk. Hosp. 91, 427-461.
6. Chambers, V. C. (1957). The prolonged persistence of western equine encephalomyelitis virus in cultures of strain L cells. Virology 3, 67-75.
7. Bang, F. B., Gey, G. O., Foard, M., and Minnegan, D. (1957). Chronic infections produced in cultured cell strains by the virus of eastern equine encephalomyelitis. Virology 4, 404-417.
8. Hearn, H. J. (1961). Differences among virus populations recovered from mice vaccinated with an attenuated strain of Venezuelan equine encephalomyelitis virus. J. Immunol. 87, 573-577.
9. Hearn, H. J. and Rainey, C. T. (1963). Cross-protection in animals infected with Group A arboviruses. J. Immunol. 90, 720-724.
10. Hearn, H. J. and Soper, W. T. (1961). Stability and correlation of virulence and plaque size in VEE virus. Bacteriol. Proc., 150.
11. Hearn, H. J. and Brown, A. (1959). Induction of increased Venezuelan equine encephalomyelitis virus synthesis in chronically infected L cells. J. Bacteriol. 78, 595-596.
12. Wagner, R. R. (1963). The interferons: Cellular inhibitors of viral infection. Ann. Rev. Microb. 17, 285-296.

DISCUSSION

Dr. Morris:

Dr. Hearn, is VEE easily transmitted in this manner, or just in this single instance?

Dr. Hearn:

No, we have done this repeatedly, the technique simply being, as far as mammalian cultures are concerned, to let the lysis take place as would occur and simply to feed by replacing cultured medium and allowing the remaining viable cells that are present to repopulate the flask.

If this flask is kept for a long enough period of time, and I might add here too, the emergence of variant attentuated population has occurred at different intervals in age of the culture, but eventually, we have found, as I say, repeatedly, that such a population does in fact emerge.

Dr. Hotchin:

I am very interested in the remark that new cell life will apparently affect the life of the culture or the life of the virus in the flask.

Do you know after you give treatment, does the virus come out in such a way that would indicate infection of all the other cells, or could it be that you are actually inducing a provirus in the cells? I wonder whether you have tried ultraviolet radiation and then immediately after that, go into infection center counts and see if you can indicate that the virus is coming out of cells that previously carried the ability to make virus, without actually making any?

Dr. Hearn:

Yes. Then, that is a moot point, Dr. Hotchin, and we had hoped to be able to shed some light on it by introducing specific antiserum into the culture.

For example, in the hopes that upon irradiation under these circumstances, that any virus that would be released would be immediately, in a sense, captured by the hyperimmune serum that was present in the environment. This would mean then that if all the cells showed lysis under these conditions then, presumably, the provirus was in state in the cell and that the ultraviolet succeeded in simulating it, resulting in complete degeneration of the entire cell sheet.

We attempted to do this also with glutamine deficient systems. I regret to say, however, that antibody has not clearly defined the situation because it offered questionable help in the ultraviolet situation. It seemed to cause a delay in the lytic process when the glutamine deficiency test was employed.

I don't think we can make any real statement concerning this point.

Dr. Stamp:

May I just make one or two comments with regard to scrapie and the present suggestions.

I think the idea of latency in scrapie is possibly one way around our difficulties regarding the question of the resistance of the agent and so on. We have done quite a lot of work in which we have tried to reveal a latent scrapie virus in sheep. To do this, we have taken considerable numbers of brains from animals which we knew were in contact with scrapie-affected sheep but were themselves normal.

We injected this brain material along with boiled scrapie brain material into young sheep, the idea being that the boiled material might stimulate a latent virus and we might get a bigger positive take than if boiled material alone had been injected.

We have also carried out similar experiments with goat material into sheep and into goats, again with the idea of trying to stimulate a latent virus. We have also used cortisone trying to see if we can produce more scrapie in normal sheep than we would expect naturally. In none of these experiments have we been successful in demonstrating latency.

We have also carried out similar experiments with tissue cultures for the reasons you have suggested. We have acted on cultures of scrapie brains and scrapie spleens, with x-rays and ultraviolet rays. We have tried to demonstrate interference, to see if the agent of scrapie would interfere with some encephalytic viruses in culture. We have tried to simulate a latent scrapie virus with such things as polio vaccine and with another virus which had been killed. All such experiments have been completely negative.

I merely make these points to show that we are well aware of the idea that scrapie virus may be latent in our sheep population, to a much greater extent than we are aware, However, it doesn't seem to matter what we do, only scrapie material will produce scrapie and we have not in any way succeeded in revealing a latent virus.

Dr. Hearn:

We have not been able in any way to demonstrate this by direct means in vivo either. We have tried the usual gamut with cortisone, x-ray, methotrexate, and so on.

Dr. Morris:

Is there anything directed against the parent strain, against the continuing strain? Will they both neutralize to the same extent?

Have you modified the antigens that have been measured by the antibody technique?

Dr. Hearn:

Our interests have not been particularly directed toward a really good qualitative examination in this respect. So that I can only give you a superficial answer to that, so far we have found that antiserum prepared against either antigen, is extremely effective in neutralizing, in the serum neutralization tests in animals or tissue cultures, the opposite strain, and so far, we have had no evidence that there is any gross difference, at least, in neutralizing capacity.

THE HEREDITARY MECHANISM IN THE CAUSATION OF NATURAL SCRAPIE IN SHEEP. II. OBSERVATIONS ON ONE BREEDING GROUP OVER 10 YEARS

H. B. Parry [1]

The source of the data and the safeguards employed in its collection have been described in Paper No. 8.

FAMILIAL RELATIONSHIPS

In low-incidence flocks affected individuals appeared without any apparent relationship to one another and in different family lines, but with adequate records, these apparently isolated cases were found to have some individual in their pedigree either affected or with a close relative affected. Affected individuals tended to be the progeny of a very small group of ewes and rams, and the ewes had a sire or grandsire in common. A ewe producing an affected offspring by one ram, often produced unaffected progeny by other rams at previous and subsequent matings, although the flock incidence remained similar. The unaffected progeny of such a ewe not infrequently had offspring which manifested the disease. Figure 1 illustrates this situation; note the low flock incidence with the higher birth group attack-rate.

In high incidence flocks many affected individuals had affected parents or progeny affected. Figure 2 illustrates the build-up of cases in a family line over 6 generations.

In one flock (flock A), the records have permitted more critical study of the family relationships of 98 affected animals. The disease manifesting itself in this flock is quite typical of scrapie as encountered in the flock-group. Losses from causes other than scrapie are low, while the productivity of the flock is high and the flock has an excellent record at agricultural shows. Thus flock A would appear to be fully representative of this flock-group.

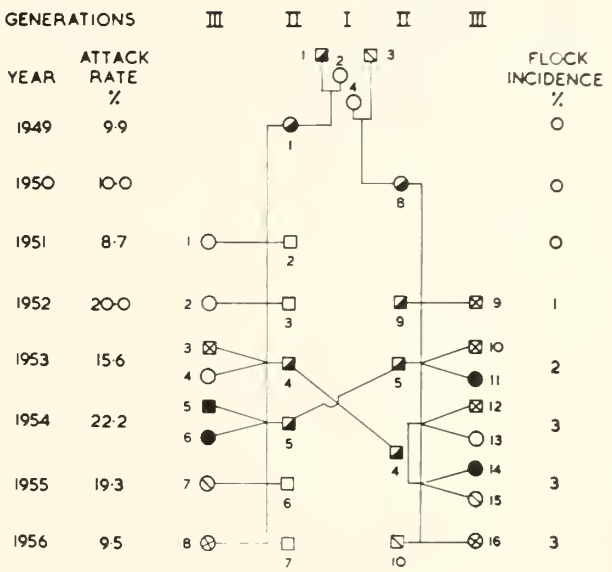


Figure 1.--A diagram to show the relationships of the first cases of scrapie known to have occurred in a free flock, 1952-56. The second column on the left refers to the attack-rate among the birth group of females form in each year and retained in the flock. The symbols are explained, as follows:

■ ● Male:female affected, i.e. ss.

◪ ◑ Unaffected, but with more than one affected progeny or a parent affected, i.e. Ss with 10 per cent. ss.

⧅ ⧄ Unclassified, i.e. unaffected but not under observation until 4½ years old.

□ ○ Unaffected with no parent or progeny affected, i.e. SS or Ss.

⊠ ⊗ Not retained on the flock, i.e., beyond 9 months of age, and not under observation thereafter.

In flock A, of 826 breeding females introduced into the flock between 1937 and 1957, 98 had manifested the disease by 1959. 50 rams have been used; 49 have been purchased from 20 other flocks, of which 14 are known to have had the disease seriously at the time the rams were born and reared, 4 are known to have had very little or no scrapie and the status of 2 is unknown.

[1] Nuffield Institute for Medical Research, University of Oxford, Oxford, England.

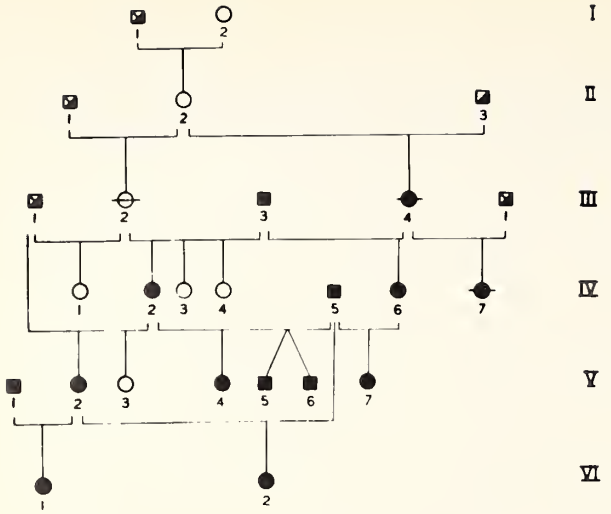


Figure 2.--A pedigree to illustrate the buildup of cases of scrapie over six generations following the introduction of affected rams.

Fifteen of the 45 rams were used for 3 seasons or longer, and left many offspring retained in the flock. Of the 15, 4 manifested scrapie, 3 others produced affected progeny when mated with affected ewes, i.e. ewes either affected during that gestation, or subsequently manifesting the disease, 3 more sired affected progeny and against 6 there is no evidence of close relatives being affected.

The remaining 30 of the 45 rams were retained for less that 3 seasons, in the majority for 1 season only and were then culled as being unsatisfactory sires, leaving few offspring in the flock. Their proneness to scrapie is therefore undetermined. Of these, 4 produced affected offspring and against 22 there is no information about close relatives.

Of the 98 females manifesting scrapie, the sires of 89 are known; 23 were the progeny of affected rams, 51 were sired by rams which produced affected progeny out of ewes which became affected, 13 were sired by rams siring more than 1 affected progeny and 2 cases were by rams siring a single case. Of the females the dams of 34% of the cases were themselves affected, while of the 35 daughters of affected ewes retained in the flock 'to 90% risk', 32 were affected. 7 affected ewes produced 2 or more affected progeny.

In matings in which the sire later manifested the disease but the ewes did not, 19 female progeny living to 4 1/2 years have shown no sign of the disease, while of matings in which the sire never manifested the disease but the ewe did, 4 female progeny have been normal to the same age and older.

In 5 pedigrees the disease occurs in 3 consecutive generations; in 7 the disease has missed one generation between two affected individuals. There is a very high attack-rate among the progeny of two affected parents, but the progeny of a mating with one parent affected may be unaffected. The pedigrees shown in Figs. 3 and 4 illustrate these points.

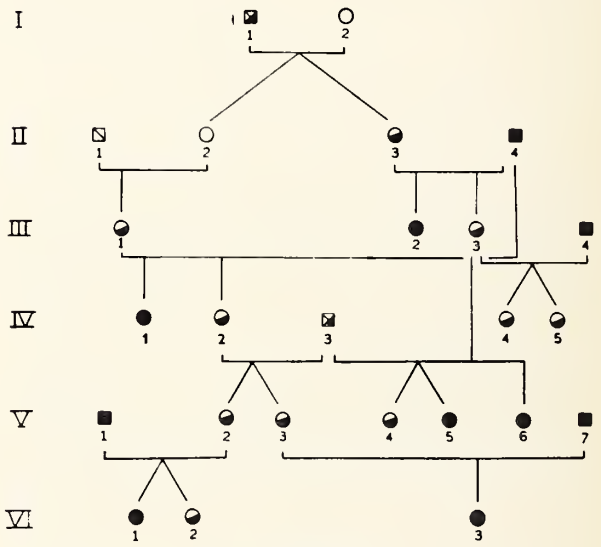


Figure 3

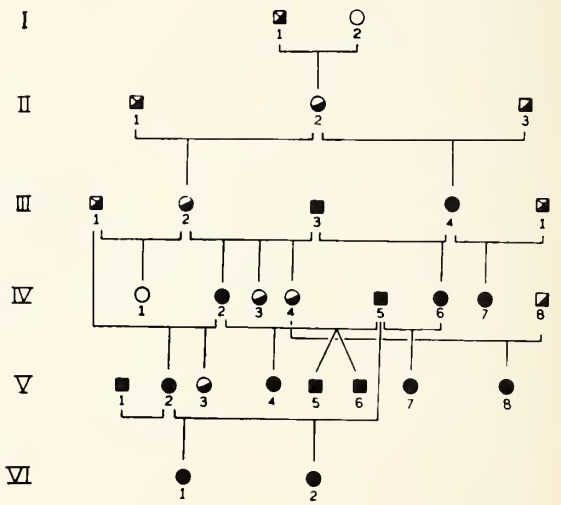


Figure 4

Figures 3 and 4.--Two pedigrees illustrating familial relationships and the increased incidence following the use of Ss and ss rams.

These data suggest that the manifestation of scrapie may well be genetically determined and that, if this hypothesis be correct, then the factor or factors are not sex-linked and do not behave as a simple dominant. The evidence could be consistent with a disease controlled by a simple autosomal mendelian recessive character.

BREEDING RESULTS

The records from 2 flocks, A and B, have been analysed for the period 1940-59. In flock A 917 breeding females and 53 stud rams have been used, and in Flock B 1108 breeding females and 73 stud rams. The stud rams have been coded on their own performance (i.e. their manifestation of scrapie or not) and that of their progeny; the records of their parents are rarely complete enough to provide any further assessment of their presumptive genotypes. With the breeding females the performance of their parents and often their grandparents, are known, information which may be supplemented in some cases by the performance of other progeny of the parents and grandparents. Thus for the dams their coding has been based on the parental performances plus their own performance, but the performance of their progeny has been excluded. The details are given below. The categories of the matings have been devised to follow the range of matings possible between the three presumptive genotypes, SS, Ss and ss. In categories 1, 2, 3, 5, 8 and 9, the genotypes of the parents may be inferred with precision, but in the categories 4, 6 and 7 the genotypes of the dams are not known precisely. The following points may be stressed: (1) The attack-rate shows a gradation from 93.8% in category 1 to 0% in categories 8 and 9. (2) the closeness of fit between the observed attack-rate for each category and that expected on the genetic hypothesis is good. (3) In category 3 the low attack-rate may prove to be due to genetically determined late manifestation, as 20 of 30 animals are the progeny of one sire, and 15 remain under observation. (4) In categories 4 and 7 there is likely to be an underestimation of Ss individuals, since they will go unrecognised unless several progeny of their matings with Ss or ss sires are kept under observation. The high manifestation in category 4 could be accounted for by preferential selection of heterozygotes over homozygous dominants, which, as we shall see later, is likely to occur. (5) In category 5 the percentage affected is too low, but the number of animals is small.

The goodness of fit between the observed figures for these two flocks and the genetic model is provisionally satisfactory. Further data providing evidence crucial for the mendelian recessive hypothesis are examined in Paper 14.

FLUCTUATION IN INCIDENCE

One of the more baffling aspects of the natural history of scrapie has been the oft-observed sudden waxing and waning of the attack-rate over a relative short period of years. The extreme polygamy with 3 males per 100 females might be expected to give such high fluctuation if a recessive factor were involved.

Preliminary studies of the effect of the use of sires of different scrapie genotype on the presumptive gene frequency of the breeding females in a flock indicate that the recessive hypothesis may well provide a perfectly adequate explanation of these epidemiological fluctuations (Draper, 1963, and paper 16). Indeed the isolated outbreak recently reported in two Indian Himalayan valleys following the introduction of exotic rams from France over 20 years previously could be readily explicable on the genetic hypothesis.

CONCLUSION

These data are qualitatively consistent with the hypothesis that natural scrapie in sheep is due to a simple autosomal recessive gene.

REFERENCES

Draper, G.J. 1963. "Epidemics' caused by a late-manifesting gene. Application to scrapie. Heredity 18, 165-171.

Parry, H.B. 1960. Scrapie: a Transmissible hereditary disease of sheep. Nature, Lond. 185, 441-443.

Parry, H.B. 1962. Scrapie. A Transmissible and Hereditary Disease of Sheep. Heredity 17, 75-105.

Figure 5

The distribution within 9 categories, based on the manifestation of scrapie in close relatives, of 82 affected sheep among 452 animals, almost all female, born into two flocks between 1945-56. Expectation is calculated on the assumption that manifestation is recessive and controlled by a single pair of autosomal alleles with late manifestation of 10 per cent.

Category	Performance of parents			Type of mating	Performance of progeny (up to $4\frac{1}{2}$ years)			
	Sire	Dam		Presumptive genotypes	Unaffected	Affected	Attack-rate (%)	
		Herself	Her parents	♂×♀			obsd.	*expd.*
1	affected	affected	...	*ss*×*ss*	2‡	30	93·8	*90*
2	unaffected with several progeny affected	affected	...	*Ss**×*ss*	4	4	50·0	*47·25*
3	affected	unaffected	one affected ; one unaffected	*ss*×*Ss*	19	11	36·6	*47·25*
4	affected	unaffected	both unaffected but one or both have affected progeny or one parent affected	*ss*× { 2·9% *ss*† ; 55·7% *Ss* ; 41·4% *SS*	25	13	52·0	*~28·3*
5	unaffected with several progeny affected	unaffected	one affected ; one unaffected	*Ss*×*Ss*	10	2	16·6	*24·8*
6	,, ,,	unaffected	both unaffected but one or both have affected progeny or one parent affected	*Ss*× { 2·9% *ss* ; 55·7% *Ss* ; 41·4% *SS*	104	17	16·3	*~14·9*
7	,, ,,	unaffected	(1) one or both unaffected with no close relatives affected	*Ss*×*SS*	7	0	4·2	*<2·5*
			(2) one or both unclassified but with no close relatives affected		161	7		
8	unaffected with no progeny affected	unaffected	unclassified but with no close relatives affected	*SS*×*SS*	19	0	0	*<0·2*
9	affected	unaffected	,, ,, ,,	*ss*×*SS*	7	0	0	*<4·5*

* 95 per cent. heterozygotes with 5 per cent. homozygous recessives (late manifestation).

† Half of the dams with both parents heterozygous, and half with one parent heterozygous and one homozygous dominant, and allowing 5 per cent. of the heterozygotes to be homozygous recessives (late manifestation).

‡ One died affected at $7\frac{3}{4}$ years and one died unaffected at $5\frac{1}{2}$ years.

DISCUSSION

Mr. Parry:

I think that Dr. Anderson asked, with regard to figures of rather high incidence in the two flocks, this does occur, but let me put it in perspective.

Within this breed and this breeder group, these are exceptional. They occur. We have an equal number with none at all. And some with a low incidence, as set out in the Heridity paper.

I don't want you to infer that all the flocks are like this. They were in fact, I suspect, if we look at Mr. Draper's curve, in a phase of an explosive "epidemic". This is pure supposition.

If I may refer to the point with regard to a tendency to select in favor of scrapie-affected animals, either the grays or blacks, this is only applicable to the Suffolks that I am working with and it is largely subjective evidence insofar as it is very difficult to measure quantatively, but it is very widely held by my flock masters. And the point is as follows: that it was brought to our attention in 1958 that a champion lamb, one of the best in that year, was very likely going to be a black. In fact, I said it was going to be a black, and it wasn't to be sold. Many of the shepherds discussed this, and its owner said it wasn't to be sold.

Now, this involved a lot of money, something on the order of $3,000, and that is a lot of money for one of our rams. It did in fact develop scrapie 3 1/2 years later, as predicted.

On numerous occasions since, in which a black ram has been used even in the best of our flocks, the daughters and sons of that ram, that is, which on our working hypothesis must be gray, have been selected as outstanding examples of the breed. They are well muscled, well fleshed, and have very firm backs, if I may put it in a non-sheepman's way instead of a professional man's way, and they have other desirable breed points, which I won't attempt to go into.

In point of fact, these animals tend to be selected even in the best flocks and we have had a number of instances, in which these daughters, rather than sell them, or destroy them when we have known that they are likely

to be at least gray, have been put on another farm under the same ownership and the approximate prediction of the number of cases has been realized. One case comes to mind, of something like 25 daughters of one ram, which we culled long before they were showing any signs, because of the father going with scrapie. I think 18 of them went down with scrapie by the age of 4.

Last year the two leading pens of females on our show circuit were in fact blacks. We knew this. They were kept deliberately and with the knowledge that once the show season was over, they would be culled; they might have been kept in the flock, because we don't bother to isolate, but at least no progeny from them would be kept for breeding purposes.

I merely quote these as a small number of instances. There are a number more. These are merely clinical impressions, but it is very widely held by sheepmen this is so, my sheepmen, and I have had enough experience perhaps to realize that when they have an idea, even if they don't know why, that there is usually some good reason behind it.

We do know that we therefore think that in general, given no other disturbance, that flockmasters at least in my group, will tend to select the grays anyway, and possibly the blacks, and we now know that some rams that have gone to Scotland from some of our flocks are in fact grays, and highly suspect. One isn't quite certain they are black, but we should learn in a year or two.

Dr. Dickinson:

Mr. Parry's understanding of the nature of evidence must differ widely from mine. The evidence you have presented is that some sheep can get scrapie and this I think many people knew already. I fail to see that this is worthy evidence applicable to anything you have said. There are sheep, too, which are highly sought after and which sell at high prices and which don't get scrapie, but if you hadn't said what you have just said, I wouldn't dream of quoting that example. But it doesn't mean anything, either.

Mr. Parry:

It depends how much you know about your sheep, Dr. Dickinson. And there are various grades of evidence. No evidence is absolute. But often there are little bits of evidence.

I am not saying this is infallible. But this points in a certain direction and so far as our breeder group is concerned, we are satisfied that this selection operates.

Dr. Dickinson:

I am sorry. I am not sure which the "this" is that does the pointing. I mean, for all I know, the evidence when examined in more detail would be that proportionately more animals that are going to get scrapie are in fact culled from the flocks.

What we must talk about here in terms of selection on growth is the proportion selected, and in terms of the proportion selected compared with the proportion culled; what are these gross proportions selected and proportions culled? What at least in these two groups are the proportions which get scrapie and it may well be that in the sheep culled, because they don't look nice or they grow, et cetera, the proportion of scrapie may be higher. This will be evidence. But just to say that some animals which look nice get scrapie, doesn't get us anywhere past the fact that some animals get scrapie.

Mr. Parry:

I think perhaps it does because in one of these high-grade flocks the progeny of affected rams tend to be selected.

I haven't got figures to put before you just at the moment, but these can be got out. Of the females born of a sire group in the flock, those that are progeny of an affected ram tend to be retained. This is without doubt.

Dr. Dickinson:

You have been working on scrapie for 10 years and so have we. It doesn't take more than an hour with plenty of records to put those figures down on paper, and you have not done it.

Mr. Parry:

It does, my dear sir, when you have such records as we have, and we haven't the facilities of your magnificant organization to work out all this data. We have to go slowly. This is a very strong impression from flock owners, and this is all I said.

I said that this is the strength of the evidence. It is not statistically verified.

Dr. Dickinson:

It is a very important point. We have heard this same rumor in the field and we don't know any satisfactory way of arriving at any understanding of whether it is a good rumor or a bad rumor. And it is important and you have concentrated on the field evidence far more than we have.

Your evidence in many cases is very good, and so you should have stacks of data on which to spend some time to produce evidence. It is the word "evidence" that is the crux of this matter. It is an important point. Somebody should be able to put out evidence if they have masses of field data.

Mr. Parry:

It will come no doubt in time. Knowledge is a very slow thing to grow.

ARTIFICIAL (POST-INOCULATION) SCRAPIE. I. THE SELECTION OF SHEEP FOR EXPERIMENTAL STUDIES

H. B. Parry[1]

In general these experimental studies can be divided into short and long-term.

The short-term studies are for less than 2 years and can be terminated before the normal age of manifestation of natural scrapie, provided the animals are inoculated young enough. In this type of experiment the accurate knowledge of the scrapie status (black, grey or white familial type) may not be of pressing importance.

However with long-term experiments involving breeding trials and comparisons of the incidence of scrapie between progeny groups over 2 years of age, the scrapie status of the initial experimental sheep becomes of supreme importance. Without complete information leading to accurate knowledge of the natural scrapie familial type of all the sheep brought into an experiment, the proper interpretation of the results may present insuperable difficulties, as it is as yet impractical to form an acceptable mathematical assessment of the likely frequencies of the 3 types in your experimental animals from the data farmers are usually able to offer.

It is only in the past 2 years that authenticated supervised records have become available for a long enough period for a small group of flocks in Britain, which now form closed breeding populations and are in a position to provide sheep of a simple definable scrapie standard for research purposes.

The details are given in Table 1.

SOURCE FLOCKS WITHOUT AUTHENTICATED RECORDS

Many long-term experiments using sheep from Group A flocks are open to serious objection on the grounds that it is as yet not

Table 1.--Assessment of Scrapie Status

Type of organization		Information available				
		Parentage	Individual identification	Production records	Health records	Grapevine intelligence
A Non-authenticated records submitted by sheepmen	1. Unregistered	None	None	Scanty	Scanty	Unreliable
	2. Registered	Sires only	Sire groups only	Some	Scanty	Usually misleading
	3. Pedigree registered	Sires and dams	One permanent system	Some	Incomplete written	Sometimes revealing
B Sheep Development Association breeder groups. Authenticated records kept under independent scientific supervision	British Sheep Society, class 3 4. Partly open breeding population 5. Closed breeding population 6. Closed breeding population with testmating for scrapie trait	Full and accurate written records of: (Rides (Mating dates (Ram movements (Raddle changes (Lambing dates (All lambs born	At least two permanent systems Tattooing Ear notching Ear tags Horn brands With temporary fleece numbers	Extensive with performance and progeny testing	Comprehensive with special note of any neurological illness	Not required

[1] Nuffield Institute for Medical Research, University of Oxford, Oxford, England.

possible to be certain whether any case of scrapie which may occur is due:

1. To the special circumstances of the experiment (and to contagion) or
2. To the inherent familial proneness to the disease (the familial type).

The validity of the inferences drawn from such experiments, which depend on the assessment of the incidence of scrapie in sheep within the manifestation age range of natural scrapie, must be scrutinised with especial care and the inferences based on them accepted with caution.

SOURCE FLOCKS WITH AUTHENTICATED RECORDS

For long-term inoculation studies of the artificial disease it seems most desirable to use sheep selected on the bases of a stricter assessment of their natural scrapie status. This is being attempted as follows:

1. Sheep must come from category B (see Paper No. 10) and from flocks of types 4 and 5 (Table 1). Wherever possible the source flocks are of type 5; test-mated sheep from type 6 flocks would be preferable, but they are not yet available in sufficient numbers. For our earlier experiments, in 1957-1959, animals not qualifying even in category B had to be used.
2. After 10 years' epidemiological study, the category B flocks can be assessed further as shown in Figure 1. For our normal controls, animals of class SC1 and SC2 of types 4 and 5 are used, while if we have used a class SC 3 animal, then only if it is from a D flock.

It must be stressed that these assessments are entirely provisional and are subject to reappraisal whenever new information becomes available. They are the best that can be accomplished in the absence of any specific laboratory test for the detection of prospective cases of natural scrapie in sheep.

General assessment of scrapie status of breeds and breeder groups.

Class		Occurrence 1952-64				Remoter memories	Assessment of scrapie status
		Supervised breeder groups in sheep			Other reports by reliable witnesses		
		Home	Bought	Sold			
SC 1		0	0	0	0	0	Breed completely clear with presumptive gene frequency zero
SC 2		0	0	0	1-2	uncertain	Breed probably mostly clear with presumptive gene frequency in supervised flocks very low but gene present elsewhere
SC 3		+		+	+	+	Breed affected with some flocks affected severely and many free Presumptive gene frequency very variable, high in individual flocks.
Individual flocks	A	+	+	+			
	B	+	0	+			
	C	0	+	0			
	D	0	0	0			
Test-mated	E	0	0	0			Declining gene frequency becoming negligible in three generations

Figure 1

ARTIFICIAL (POST-INOCULATION) SCRAPIE. II. THE RESPONSE OF SHEEP TO INTRACEREBRAL INOCULATION

H. B. Parry [1]

DONOR ANIMALS FOR INOCULATION

Natural cases of scrapie in sheep have been selected with unequivocal signs of classical scrapie of stage 3a, obtained from supervised flocks showing the familial and hereditary pattern of occurrence.

THE INOCULUM AND INOCULATION

In every experiment the inoculum has been freshly prepared from the brains of a new natural case and a normal sheep. The normal and then the affected sheep has been killed by Pentobarbitone given intravenously, the brain exposed in a sterile manner, half the brain used in preparing a 1/10 $^{w}/v$ homogenate in saline and the other half being fixed for histological examination. All glassware has been cleaned in 'Chromic acid' mixture before sterilisation by autoclaving; all instruments have been cleaned thoroughly in detergent solution before autoclaving, while cutting instruments have been new.

The supernatant from light centrifugation of the homogenate without addition of antibiotics has been injected into the recipient animals the same day, or occasionally up to 36 hours after its preparation with storage over-night at 1^{o} C. The inoculation was done under light pentothal anaesthesia into the left parieto-occipital region of the brain.

Normal sheep of breeds & breeder groups free of scrapie, (see paper No. 18) have been used of about the same age, sex and parity.

RECIPIENT SHEEP

Some have been born on our own experimental farm in a contaminated environment. Some have been purchased at varying ages, mostly 6-18 months old, from supervised flocks with authenticated records, wherever possible. Apart from the newborn lambs of either sex, the older sheep have been females & bred from during the course of the experiment.

GENERAL RESULTS

No sheep has developed clinical signs in less than 12 months nor longer than 24 months so far, although there are a number of survivors among animals inoculated over 3 years ago (see below).

In one instance a clinically normal sheep of an affected flock-group but from a flock at that time free of the disease (and for the previous 5 years) was used as an inoculation control. The histological examination showed probable early evidence of scrapie, later some of the sheep inoculated developed the disease within 15 months and before 18 months of age, and one year thereafter scrapie occurred in close relatives of the parents, & siblings, which made it probable that the sheep could have been developing natural scrapie.

AGE AT TIME OF INOCULATION

New Born Lambs

The lambs, born in 1957, were 4 to 24 days old and suckling their dams, which were of 2 breeds; the sires of the lambs were of 2 breeds. The sires and dams were normal and thought at the time to be clear of scrapie, but in the light of recent knowledge, their presumptive genotypes are now less certain. In 2 of the 3 breeds of the parents, scrapie is

[1] Nuffield Institute for Medical Research, University of Oxford, Oxford, England.

probably extremely rare, but in the third it does occur. Of 10 lambs, 5 were inoculated and 5 were left uninoculated as controls, each paired as far as possible for weight, sex, age and parentage, and reared together on their dams in the open.

The 5 inoculated lambs developed clinical signs of a scrapie-like illness, in the females at 12, 14 and 22 months and in the males at 13 and 14 months after inoculation. One control animal was killed at 17 months for comparison with its fraternal twin which had been inoculated; the remainder were alive and well at 30 months.

Age 1 1/2 to 8 years

Some results are shown in figure 1. We also have an experiment in hand using a breed found by Gordon (1959) to be one of the most highly susceptible; no case has yet occurred after 21 months among 20 animals inoculated when 12 months old, yet mice inoculated at the same time have all gone down at 15 to 18 months.

Effect of inoculating female sheep of breeds free of natural scrapie

(Intra-cerebral, from single genetic case, October 1960)

Breed N°		71			75			77		
Age in years		8			1½			1½		
Nature of inoculum		S	N	C	S	N	C	S	N	C
N° inoculated		2	1	0	8	5	4	12	8	5
Losses in -										
1st yr	Scrapie	-	-	-	-	-	-	-	-	-
	Other	-	-	-	-	1	-	-	-	-
2nd yr	Scrapie	-	-	-	⑤	-	-	④	-	-
	Other	-	-	-	1	-	1	1	-	-
3rd yr	Scrapie	-	-	-	-	-	-	-	-	-
	Other	-	-	-	1	-	1	1	-	-
Survivors after 3⅓ years		2	1	0	1	4	2	6	8	5

Figure 1

BREED

Figure 1 shows the results of inoculating sheep of 3 breeds considered to be in Class SC1 (Paper No. 44). They are very variable but are in general agreement with Gordon's (1959) results.

NATURAL SCRAPIE GENOTYPE AND SUSCEPTIBILITY TO THE POST-INOCULATION DISEASE

The natural scrapie gene frequency of the 3 breeds shown in figure 1 are probably very low; the chances therefore of these inoculated sheep carrying the scrapie gene are small and for practical purposes (until such time as test-mated stock are available) may be taken to be nil., i.e. all these sheep are of SS genotype.

It will be seen that some of these sheep are much more susceptible than others, i.e. there appears to be no close relationship between susceptibility to the artificial disease and the presumptive genotype of the natural disease.

Gordon's (1959) results also show in many breeds considerable divergence between susceptibility to the artificial disease and their known liability to the natural disease. However it is impossible to draw any firm conclusions from his data since no reliable information is available about the natural scrapie genotypes of his animals.

CONCLUSIONS

The response of sheep to inoculation with material from sheep naturally affected with scrapie is very variable (as others have shown).

It would appear to be dependent on at least the following:

(a) Donor's breed and individuality.

(b) Recipient's age and breed.

A response can be obtained from a preclinical donor in which histological evidence of early brain degeneration was present.

Sheep of white familial type in regard to natural scrapie may or may not be susceptible.

The data strongly suggest that there is no close correlation between susceptibility to artificial (post-inoculation) scrapie and liability to the natural disease.

The use of current inoculation procedures to determine the susceptibility or liability of a strain or family line of sheep to natural scrapie offers little chance of successfully distinguishing between the three natural presumptive genotypes (black, grey and white).

REFERENCE

Gordon, W.S. 1959. Scrapie Panel. Proc. 63rd Ann. Meet. U.S. Livestock San. Assoc. p. 291.

DISCUSSION

Dr. Stamp:

I might make the point that these sheep, both the Dorsets and South Downs were purchased in the South and were only kept in Scotland for a comparatively short period before they developed scrapie. They were actually English sheep and were almost certainly infected before they came to Scotland.

Mr. Parry:

All I want to point out is that it seems to me that susceptibility to the artificial inoculated disease does not appear to be very closely related to the genotypes controlling the natural scrapie disease about which Draper and I have been talking. We are dealing in this experiment with animals which are almost certainly white, although not proven white by test-mating and some of them are susceptible to the post-inoculation disease and some are not.

We cannot yet do the critical experiment in this because we do not have test-mated animals, proven clear of the scrapie gene, whatever it may be, and it is not possible to have these animals available for five years. Hence, we can't take the matter further.

We think it unlikely that the inheritance to the artificial disease and the inheritance to the natural disease are closely related, and we consider it wiser to keep these two diseases separated in our minds.

I may have to modify my views tomorrow after I have heard what has been said.

Dr. Dickinson:

Could I take up the point about the Cluns. Going back to the film this morning--that last animal shown, was it not a Clun?

Mr. Parry:

No, it wasn't.

Dr. Dickinson:

What was it?

Mr. Parry:

I will tell you in private.

Dr. Dickinson:

This question of the disparity between the incidence of natural scrapie and the apparent inducibility of the disease, the contrast is most strongly brought out in the Herdwick case which Dr. Gordon chose to use, because on one small sample he got 78 percent incidence of the induced disease whereas I know of only one case of scrapie in the Herdwick breed, and that many, many years ago. And I have many tabs out on the Herdwicks. I think my information on Herdwicks is as reliable as it is for any other breed beside the Suffolk. And I wonder if you have any information of natural scrapie in Herdwicks?

Mr. Parry:

Yes.

Dr. Dickinson:

Have you many cases?

Mr. Parry:

I had about five cases, about 1952. I haven't been in touch since.

Dr. Dickinson:

Here it certainly is a low incidence situation in your mind, isn't it?

Mr. Parry:

I have no idea.

Dr. Dickinson:

Low incidence naturally, with a very marked increase in the experimentally challenged ones.

Mr. Parry:

I have a very open mind about these hill breeds. I mean I don't know enough. I had been given to understand some Swaledales had scrapie, but I thought the Teeswaters were clear. I had always been told Mashams were clear. I am now not prepared to accept any such statements unless I have detailed records. You may have information that I haven't, and I have information that perhaps you haven't.

Dr. Mulhern:

Mr. Parry, you said this morning that there is a transmissible agent. Do you mean in natural scrapie?

Mr. Parry:

Yes, in my blacks.

Dr. Mulhern:

There is a transmissible agent in natural scrapie?

Mr. Parry:

Yes; although I have little experience about artificial scrapie, because I haven't done passages. There is a vast amount of work done by Dr. Gordon's and Dr. Stamp's laboratories. I accept this.

Dr. Mulhern:

So under natural situations, then, you say that there is a transmissible agent involved? Is that right?

Mr. Parry:

In my black sheep.

I will amplify that. In a previous experiment the half brain from one of my controls, what I thought was a normal Suffolk sheep, went away for histology. Six months later Mrs. Beck said, "I think this is a case of scrapie. It is showing the early histological signs." And I said, "Oh, no, it is not"--you know, very firmly--"because it comes from a flock where we haven't had any." 18 months after the histological diagnosis on this animal some of the inoculated sheep go down, which would indicate to me in my simple way that there is a transmissible agent in the inoculum. Six months later I get more evidence about this particular family line, because progeny and half sibs have reached the age of manifestation. Some of them go down with natural scrapie in the flock of origin. So my original assessment of freedom from my genetic disease, as I thought, was inaccurate.

This particular animal showed no clinical signs. It did, in fact, have early changes in mossy fibers, with an odd vacuole or two.

All I can assume, on what we now know, is that we had in fact picked a case of preclinical scrapie. It wasn't pre-pathological, but it was pre-clinical.

Dr. Mulhern:

In the clarification of this transmissible agent, it is where you have a susceptible line that something is transmitted naturally from one to another?

Mr. Parry:

We wouldn't think so.

Dr. Mulhern:

I am trying to pin down that there is a contagion that goes from one animal to another, providing the blood lines are there, the susceptible blood lines.

Mr. Parry:

We don't think this is so. But I will answer this specifically in a moment.

Dr. Gordon:

Your hypothesis does suggest that this disease is transmitted hereditarily, and only by that method. It never happens by lateral spread from affected to susceptible animals.

Mr. Parry:

We have found no evidence for this.

Dr. Gordon:

You have made this point very clear.

Now will you tell me how this disease gets from one pure breed to another pure breed; because if these are registered sheep there is no interbreeding between pure-bred breeds. How does the gene for scrapie get across from one pure bred to another if it doesn't manage to jump this gap by the infectious method?

Mr. Parry:

How do you know it gets from one pure-bred to the other Dr. Gordon? I don't know. You see, the thing is that none of us--I think we better be quite frank about this:--<u>none of us</u> know enough about the back history of scrapie in any pure breed, any in England--I don't know about Scotland--in which one can say that scrapie has never occurred in that breed, and that therefore that breed is completely free of the scrapie gene.

Let me have my gene for a moment. You can have your concept all day tomorrow.

This means that you can have a very low frequency of the gene in one pocket of the

breed, with an odd case, which doesn't get reported, or doesn't get recognized. We suspect that this happens. I have no proof for it.

If that happens, the breed in fact is carrying the gene and if you get the right matings done, sooner or later you will get cases. And at this moment I favor this explanation for the apparent outbreak in a clear breed. After tomorrow I may have to modify this entirely. But allow me to have it tonight.

Dr. Gordon:

I would prefer you to modify it tonight rather than tomorrow.

Dr. Walker:

I have to get this down in language I can understand.

Are you saying that the agent of scrapie is a transmissible agent, but it must be carried strictly through the recessive genes? In other words, if an animal has double recessive genes for scrapie, he will definitely come down with scrapie.

Mr. Parry:

Yes, every time.

Dr. Walker:

In other words, the sheep could not have recessive genes and not come down with scrapie?

Mr. Parry:

Not in our experience.

There may be situations outside of our experience. I wanted to come to this point in a moment (in paper 21).

Dr. Walker:

I would like to make one statement rather than a question. It would appear to me that, if you have a transmissible agent and there is a genetic factor involved, you could have animals carrying the double recessive gene and it would depend on the presence of the transmissible agent and not as to whether the animals would actually come down with scrapie.

Dr. Dickinson:

The point simply is that it is just not getting home. This is our difference, which is fundamental to any eradication policy. People have not understood it.

Paper No. 20

ARTIFICIAL (POST-INOCULATION) SCRAPIE. III. A PRELIMINARY REPORT ON ARTIFICIALLY TRANSMITTED SCRAPIE IN THE SHEEP AND MOUSE

A. Vince [1]
Presented by H. B. Parry

EXPERIMENTAL DISEASE OF SHEEP

Twenty-eight sheep suffering from experimentally induced disease are being examined. Each brain has been cut into seventeen slices. There has been no evidence of macroscopic change, with the exception, in some cases, of dilatation of the ventricular system. Serial sections of the cerebellum, medulla, pons, midbrain and hypothalamus have been studied, four consecutive sections being stained and fifteen preserved for future investigation. The spinal cord, peripheral nerves, muscles, and abdominal organs, as well as the endocrine glands, are being examined.

From a preliminary survey, it has become obvious that the non-specific changes, e.g., shrinkage and increased basophilia of nerve cells, hypertrophy and proliferation of astrocytes and status spongiosus, described by

[1] Nuffield Institute for Medical Research, University of Oxford, Oxford, England.

Hadlow, are by no means the only features. In addition to these non-specific changes, we have consistently found degeneration of the hypothalamo-neurohypophyseal system and the cerebellar system. It would appear that such neurological involvement varies in extent and degree, but that some animals have contracted a more severe form of scrapie than that usually occurring naturally. Otherwise, the pathalogical changes appear to be identical with those of the natural disease. The classical type of inflammatory reaction which is found in those types of encephalitis known to be caused by already identified viruses is not seen in experimental scrapie affecting the sheep.

THE ARTIFICIALLY INDUCED DISEASE IN THE MOUSE

In mice injected intracerebrally with an inoculum prepared from a sheep with natural genetic scrapie, intracellular vacuoles have been found, as well as microcysts and astrocytic proliferation, together with patchy loss of Purkinje cells in the cerebellum. We have confirmed Chandler's findings of extracellular microcysts, diffuse astrocytic proliferation and vacuolated neurones. As in the sheep, no classical inflammatory reaction attributable to any previously identified virus has been observed.

DISCUSSION

Mr. Parry:

All we say is that we get the similar lesions in these two neuroanatomical systems.

Dr. Stamp:

Which lesions?

Mr. Parry:

In the two systems, the cerebellar and hypothalamic-neurohypophyseal systems.

Dr. Stamp:

It isn't as easy as that.

Mr. Parry:

We rather think it is in the animals I have been talking about, Suffolks and Southdowns, Cluns, and Suffolk--

Dr. Stamp:

It is always the Suffolk agent?

Mr. Parry:

I have worked with nothing else.

I would agree with Dr. Hadlow: But we wonder if some of these, as we think, are secondary non-specific changes. I am not going to argue this one. There is one other point about the artificial disease about which I would just very briefly like to report, which is not complete.

We have wondered whether, just as you have tried to make your presumed latent agent come up with cortizone and so on, whether we could alter the response of our lambs by whole-body irradiation of the newborn lambs. This in fact has to be done very early in life because there is a very steep rise in LD50.

We had about 20 survivors. We took twins. They had a Welsh Mountain mother and Suffolk father. The Suffolk father I thought was clear. I now know it to be a gray. This complicates the position.

Very briefly, we have been going a little over 2 years. Of the inoculated group one of the pair which received the scrapie agent was unirradiated. Six out of six of the unirradiated animals have died. Out of the irradiated litter mate, receiving the same scrapie agent, the same day, none have died. None out of eight.

What is going to happen? We don't know. We want to irradiate blacks. We think we have one irradiated which has come down, or looks as if it is coming down at 3 years. It is too early to say. And the data isn't big enough.

All I am saying is the radiosensitivity--if I may use that in a broad sense--of the artificial transmitted disease is, I think, perhaps a little unexpected. But we want to do more work.

And there is a suggestion that the natural genetically determined disease may not react in just the same way. But this is only a hint, and I don't want to press its significance.

THE HEREDITARY MECHANISM IN THE CAUSATION OF NATURAL SCRAPIE IN SHEEP. IV. THE POSSIBLE RELATIONSHIPS OF THE TRANSMISSIBLE SCRAPIE AGENT (PROVIRUS) TO THE GENETICAL COMPONENT

H. B. Parry[1]

The evidence in favour of the hypothesis that the clinical expression of natural scrapie in sheep is controlled by an autosomal recessive gene is extremely strong. There is also good evidence that in sheep clinically affected with the genetically determined type of natural scrapie there is a transmissible agent of virus-like characteristics.

This agent (TSA) has certain properties, some of which differ from those commonly associated with the infectious viruses of the usual communicable type. These properties have been worked out mainly at Compton and Moredun and are summarised below (for references see Parry, 1962):

SOME PROPERTIES OF THE TRANSMISSIBLE SCRAPIE AGENT

Pathogenicity	Sheep, goat and mouse.
	In tissue homogenates to 10^{-5}.
	Many routes: intracerebral preferred.
	Long latent period and observe for 1-2 years.
	Not aided by adjuvants of allergic EM.
Thermal Stability	Survives at room temperature for 42 days.
	Survives in saline 99.5°C for 8 hours.
	Destroyed by autoclaving at 220 lb. p.s.i. for 30 mins.
Chemical Stability	Survives 0.35% formaldehyde for several months.
	Survives acetylethyleneimine 1 1/2 x lethal conc^{n}. for viruses.
	Survives extraction in N-butanol and N-diethyl ether.
Physical	Particulate: size between 27-410mμ.
	Diffusible into cerebrospinal fluid.
Biological	Self-replicates in suitable recipient animals.
	Non-antigenic to tests positive for many viruses.
	Cytopathogenic for CNS cells and ? muscle.
	Present in many body organs but not blood, semen etc.

The type of localised neuronal damage in the C.N.S. without evidence of inflammatory stigmata differs from the pathology of the usual encephalogenic viruses.

There are thus good grounds for the view that TSA may not be strictly comparable with the infectious viruses commonly affecting the C.N.S. and that it may be more properly considered to be an incomplete virus or provirus.

We are thus faced with the unusual biological situation of a gene-controlled disease with which a virus-like particle (provirus) is intimately associated.

SUSCEPTIBILITY TO A WIDESPREAD INFECTION

The simplest explanation might be that the recessive allele merely determines susceptibility (and its allelomorph resistance) to TSA. For this view to provide an acceptable explanation of the observed phenomena, certain assumptions become necessary:

As all cases of natural scrapie prove fatal, and the quantitative pattern of occurrence in

[1] Nuffield Institute for Medical Research, University of Oxford, Oxford, England.

the field fits the recessive gene hypothesis so closely, susceptibility (or resistance) must be complete. (Unless one postulates a very much more complex multifactorial mode of inheritance for which no clear evidence exists at present). There is, of course, no way of telling whether those animals lost from competing risks would have developed scrapie eventually. However, the number surviving to age 2 years and lost thereafter from causes other than scrapie is very small (some 5%), and hence is unlikely to affect the issue.

We know from observation that all (or at least the great majority of) animals of presumptive ss genotype manifest the disease, and the 'goodness-of-fit' of the other data to the recessive hypothesis supports this view. Thus all ss animals must meet the infection. Yet the majority of individual outbreaks occur in sheep with both parents unaffected and in flocks in which no case of scrapie has occurred for many years. Thus there must be a widespread source of infection either in parents in flocks without clinical cases or on the premises. As there is no good evidence to support mediate contagion or residual contamination as a means of spreading natural scrapie (see Paper No. 9) the presence of the infection is presumably in carrier animals, which must be widespread in Britain now and have been present in the sheep stocks of Western Europe for at least the last 200 years. Thus the TSA should be and have been an ubiquitous infection of Western European sheep for the last two centuries.

As susceptibility to TSA would have little opportunity of exerting an effective selection pressure one must assume that the ss genotype had certain advantages and by chance offers some favourable substrate, which permits TSA to be lethal in this genotype.

There are difficulties in accepting this hypothesis:

1. TSA has not been demonstrated in unaffected carrier animals (all assays of normal animals have so far been negative for TSA).
2. No specific antibody response to TSA has been detected, which one might expect even if TSA behaved as a 'hidden' virus like herpes simplex in man.
3. The infections must have been widespread in normal carrier sheep in Europe between 1750 and 1850 when many of the original importations to Australia and North America were made; it is difficult to explain the absence of scrapie in both North American and Australian sheep until about 1952, unless one assumes that none of the original importations carried the postulated recessive gene for susceptibility, which appears most unlikely.

TSA IS ENTIRELY GENE DETERMINED AND PRODUCED DE NOVO IN ss INDIVIDUALS

The evidence for this hypothesis is summarised briefly in figure 1 (Parry, 1962).

Some biological characteristics of TSA.
(Scrapie provirus).

1. Produced in sheep of ss genotype.
   (a) coinciding with earliest brain damage
   (b) at or shortly before clinical illness.
   (c) present in many tissues, but primary pathogenic effects localised to brain and possibly muscle.
2. Not produced in SS or Ss sheep.
3. After inoculation multiplication occurs in some sheep but not all
4. A diffusible particle reacts with a substrate present in many sheep to form TSA.
5. TSA does not pass naturally from one sheep to another.
6. In nature sheep aquire TSA entirely through heredity; never by infection.

Figure 1.

CONCLUSIONS

It is not possible to distinguish between these two hypotheses from a consideration of the genetic evidence, since in each case the crucial controlling factor is a simple recessive gene.

Against the susceptibility hypothesis the lack of evidence for any widespread infection of the TSA in countries where scrapie now occurs and the unlikelihood that the infection, were it present, would not have been taken to North America and Australia in the 19th century diaspora of sheep from Britain are substantial difficulties. When the gene-determined provirus theory was proposed in 1960, it was the only known example of this kind of situation among the higher mammals. Since then the demonstration of a transmissible agent in amyotrophic lateral sclerosis of man (Zil'ber, et al., 1962) which is probably hereditary, may provide an example of a similar kind in the same general biological group of neuromuscular disorders. There are also a number of recent reports on self-replicating hereditarily determined particles which may be relevant to scrapie.

With the publication of these papers, the gene determined provirus hypothesis seems less bizarre. It provides the more reasonable explanation of the published data on scrapie presently available.

REFERENCES

Parry, H.B. 1962. Scrapie: a transmissible and hereditary disease of sheep. Heredity, 17, 75-105.

Zil'ber, L. A., Baidakova, the late Z. L., Gaodash'yan, A.M., Konovabov, N. V., Bunina, T. L., and Banabadze, Ye. M. 1962. (Possible virus aetiology of amyotrophic lateral sclenosis). Vopnosy Virusologii, 5, 520-528.

DISCUSSION

Mr. Parry:

May I just go on to very briefly consider this question of whether this is a susceptibility to widespread infection or not. It has been mentioned before, so I will be very brief.

I think Dr. Dickinson made the points I wanted to make, that if this gene is simply a genetic mechanism of susceptibility or resistance, you have to postulate an ubiquitous infection; so that every case of the double recessive genotype in the flocks I have been dealing with, must come up against the infection because they all go down, and the data fit the recessive hypotheses so well, in so many cases.

The first case in a flock is so frequently an animal with both parents unaffected, and this presupposes that the infection or the infective agent must be present in that flock in some form.

However, the difficulty at the moment is that nobody has demonstrated the presence of this agent, except in clinically affected sheep. This may be, as Dr. Stamp suggests, a matter of latency or something of this kind. Until we have evidence of this, I am merely putting up this theory because it fits in my view the evidence as we have it at the moment.

It is odd that we have no specific antibody response, if it is a persistent infection like herpes in man or even like the murine lymphocytic chorio-meningitis that was spoken about.

There is a further point which Mr. Draper made, that on our data you cannot distinguish between the two possibilities of total susceptibility to an agent on Dr. Gordon's lines, or my hypothesis that it is a gene responsible for a specific degenerative process in the central nervous system--like the heritable cerebellar ataxias or other disorders in man--and in which, just before clinical illness occurs the transmissible agent can be demonstrated.

We will just have to wait and see what the future brings forward. But from a practical point of view it is of some interest because it could lead to certain different conclusions as to what is the appropriate means of containing these scrapie syndromes.

Dr. Gordon:

Mr. Chairman, I would like to refer to Mr. Parry's statement that scrapie can only be transmitted from clinically affected sheep.

Yesterday, I made it clear that of 114 sheep, whose tissues were used to make louping-ill vaccine, 8 were believed to be in the incubative stage of a scrapie infection. Alternatively you might suggest they belonged to your black category but although none was showing clinical signs they effectively contaminated a batch of louping-ill vaccine with scrapie. Can brain material from one of your classified blacksheep, not yet showing clinical signs, produce when inoculated into a susceptible animal?

Mr. Parry:

We would love to do it and are planning to do it. We haven't done it for two reasons. One is the blacks have been too valuable to establish ratios, and statisticians won't allow me to kill off my blacks.

And, secondly, we haven't got enough animal accommodation, but if you would be kind enough--

Dr. Gordon

I would be delighted to be presented with a black that you predict is going to take scrapie, so that I may have its brain for transmission purposes in the hope that I may persuade you that scrapie can be transmitted from animals now showing clinical symptoms.

Mr. Parry:

I have just the thing. Did I understand Dr. Stamp a right to say that you had taken formalin fixed material?

Dr. Gordon:

Correct.

Mr. Parry:

We obtained a fetus, 44 days old. It is a black. It is in formalin.

Dr. Gordon:

It doesn't matter.

Mr. Parry:

You shall have this. I am perfectly happy to help if we can come together on this, because I cannot hold any more animals on my limited facilities; therefore, I have to put back these experiments that I feel ought to be done.

Dr. Gordon:

I will happily arrange this if you will let me have the material.

Paper No. 22

REVIEW OF THE AVIAN LEUKOSIS COMPLEX

B. R. Burmester [1]

When I was invited to give a talk here on leukosis, I wondered why the people attending a seminar on scrapie would be interested in my giving a paper on Avian leukosis, but after sitting in the audience and listening to the remarks, ideas, and contradictions thrown back and forth here this morning, I feel at home. It reminded me of many meetings that I sat through discussing the leukosis complex.

One of the highlights occurred in 1908, when Ellermann and Bang were able to produce the leukosis in chickens with a filterable agent, yet even up to 1940, eminent pathologists claimed up and down, in textbooks and otherwise, that lymphomatosis was not transmitted by a virus. So, we have many things in common with research on scrapie.

The diseases of the avian leukosis complex, and I refer to them as plural because they really are different infectious agents causing various types of neoplasms.

The infectious agents also are in my definition, contagious. But this varies considerably, depending on the type of neoplasm that we are talking about.

[1] B.S., M.A., Ph.D., D.V.M., Animal Husbandry Division, Agricultural Research Service, U.S. Department of Agriculture, East Lansing, Michigan.

Now, as far as history is concerned, it was back in 1896 that Caparini first described avian leukosis, and in 1905, Butterfield, in Michigan, described the lymphoid tumor which is part of the avian leukosis problem.

Then in Germany, Dr. Marek described what he called fowl paralysis, which we consider as part of this complex. As I indicated, in 1908, Ellermann and Bang definitely demonstrated a filterable agent which caused leukosis.

And then in 1909, Peyton Rous found a filterable agent which caused fibrosarcoma. I am going to use various figures and tables to illustrate my talk.

Listed in table 1, are the various neoplasms that have been demonstrated to be induced by filterable agents.

There are three types of leukosis, erythroblastosis, myeloblastosis, or visceral lymphomatosis, reflecting the three types of principal blood cells.

The first two are always leukemic, whereas the lymphoid leukosis is generally aleukemic, and then we have the neural and ocular lymphomatosis, which also involves the lymphoid cell, but shows slightly different pathologic and epidemiological picture.

Myelocytomatosis, is a chronic type of myeloid involvement, and then we have what is called the related neoplasms, osteopetrosis and, some people still consider osteopetrosisas not a neoplasm, fibrosarcomas, histiocytic sarcomas, and endotheliomas, nephroblastomas, osteochondro sarcomas, chondromas, and then we also get hemorrhagic lesions which are related to the endothiliomas.

Table 1.--Virus induced neoplasms of the fowl

Name	Code	Synonyms
Leukosis:		
1. Erythroblastosis.....	Ery	Erythroleukosis
2. Myeloblastosis.....	Myel	Granuloblastosis
3. Visceral lymphomatosis....	VL	Lymphoid leukosis Lymphocy oma
4. Neural lymphomatosis[1] [2]...	NL	Fowl paralysis
5. Ocular lymphomatosis[1] [2]...	OL	Iritis
6. Myelocytomatosis[1].....	Mycy	Myelocytoma
Related neoplasms:		
7. Osteopetrosis[2].....	Ost	Marble bone
8. Fibrosarcoma	Sar	
9. Histiocytic sarcoma.....	His	
10. Endotholioma	End	
11. Nephroblastoma.....	Neph	Renal odenocarcinoma
12. Osteochondrosarcoma		
13. Chondroma	Cho	
Related lesion:		
14. Hemorrhage, hemotoma	Hem	

[1] Unproven etiology.
[2] Not considered neoplasms by some investigators.

Lymphomatosis, after its first description, did not become of economic importance until after large scale commercial type management practices were introduced into the poultry industry, especially the use of the incubator and the large brooder unit.

So, in the early 1920s, we find the occurrence of this disease in more or less epidemic proportions in commercial flocks. The data on Figure 1, show the change in the relative occurrences of the two forms, neural and visceral lymphomatosis in a laboratory in California. The records from '31 to '40 show a general decrease in the neural form and a general increase in the visceral form.

Table 2 shows the comparative data in the laboratory flock for 13 years and in the Cornell flock for 5 years.

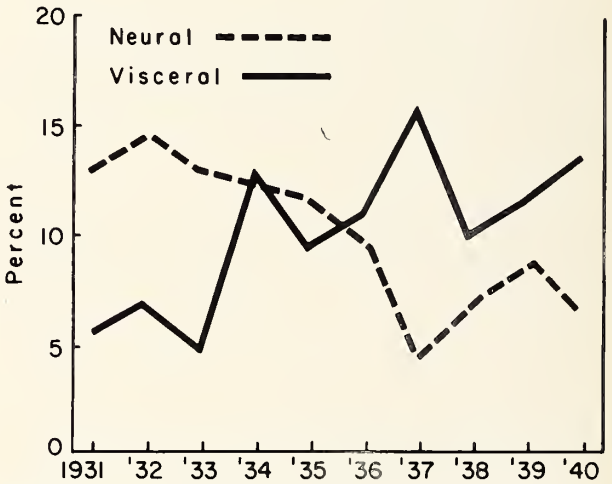


Figure 1.--Occurrence of neural and visceral lymphomatosis in California (modified from Hoffman and Stover, 1942).

Table 2.--Distribution of different types of lymphomatosis in two flocks

Flock period	R.P.R.L. 13 years	Cornell Expt. Sta. 5 years.	
Genetic select.	All lines	Resist.	Suscept.
Tot. No. chickens...	11,823	9,417	2,977
Percent neoplasms...	29.4	5.2	52.1
Distrib. of neoplasms.....			
Lympho. - visceral..	72.0	51.9	14.4
- neural....	23.2	32.7	80.7
- ocular....	2.8	3.9	2.1
Other neoplasms.....	2.0	11.4	2.7

The lymphoid disease, lymphomatosis, is the only one of the various virus induced neoplasms which occurs at a high rate under natural conditions and is important from an economic standpoint.

Research workers at Cornell, for a number of years, have been selecting for resistance and susceptibility to this complex. We find the distribution of the neural and visceral forms to be quite different for the two strains of chickens. In the resistant line there is a high proportion of the visceral form, whereas in the susceptible flock a high proportion of the neural form.

I want to emphasize that these figures are not the percent incidence, but they are the percentage distribution of these types of neoplasms.

Data in Figure 2 show the age distribution of the various types at the R.P.R. Laboratory. The neural form occurs early, and the visceral form definitely has a peak after the neutral form, but mortality continues for a long period into the older age groups. A combination of the visceral and neural form also occurs, and at least in this flock, the ocular form was very sporadic. This is a typical picture that is seen in commercial flocks with the neural occurring early and the visceral starting at sexual maturity and continuing for a long period.

Table 3 gives the data on the occurrence of early high leukosis mortality in a test, where many chickens were brought in for testing of production activity. This high mortality at an early age here is given in weeks.

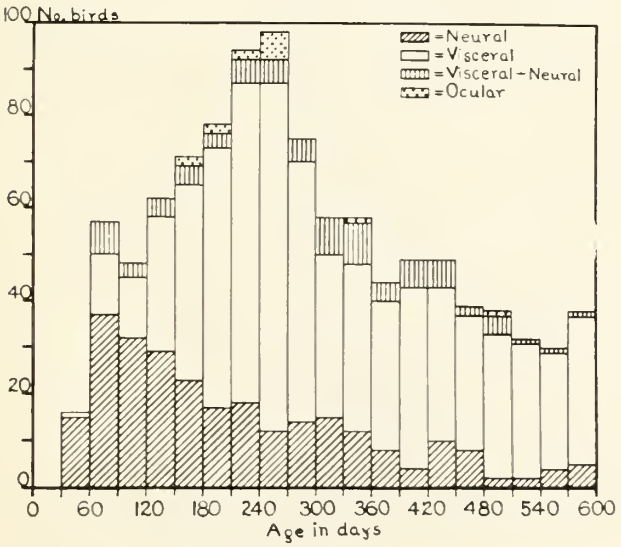


Figure 2.

Table 3.--Pennsylvania random sample test
48 entries - 62 chicks per entry

(Gentry and Seitz,

Chicks started May 3, 1960.....	2,976
Chickens alive Oct. 1, 1960.....	2,222
First 22 weeks mortality.....	754
Percent mortality.....	25.3
Pullets housed Oct. 1, 1960.....	2,058
Pullets alive Feb. 28, 1961.....	1,940
Second 22 weeks mortality.....	118
Percent leukosis mortality.....	3.0
Percent other mortality.....	2.7

At 13 to 16 weeks, there was a very high peak in mortality, the chart represents total mortality, but actually it is over ninety percent lymphomatosis.

Now just a word with respect to the nature of the causative agent. It is, of course, a virus, an RNA type of virus with diameter of approximately 80 millimicrons.

Figure 3 shows sections of the virus, definitely showing an electron dense core which is approximately one-third the total diameter.

Figure 4 shows a section of a tumor, and this is highly selected for high virus population. This is actually a kidney tumor, a nephroblastoma, induced by a strain "A" virus. Note these particles in between the cells, and also notice the budding of the virus particles off the cell surface. This is the principal and perhaps the only way the virus particle becomes mature. There is a condensation of the ribonucleic acid in the cytoplasm, forming the nucleoid and then, this more or less protrudes on the cell surface, and is pinched off taking with it apparently some of the cell membrane.

The virus ends up with a little part of the cell protein on the surface of the virus. All those who have worked on this phase agree that virus from all the various strains that have been studied are indistinguishable from a standpoint of the physical structure.

Now a word with respect to the biological nature: Virus that produces some forms of leukosis has been studied with relative ease, especially those forms of the disease like erythroblastosis and myeloblastosis, which are not generally found under natural conditions of infection.

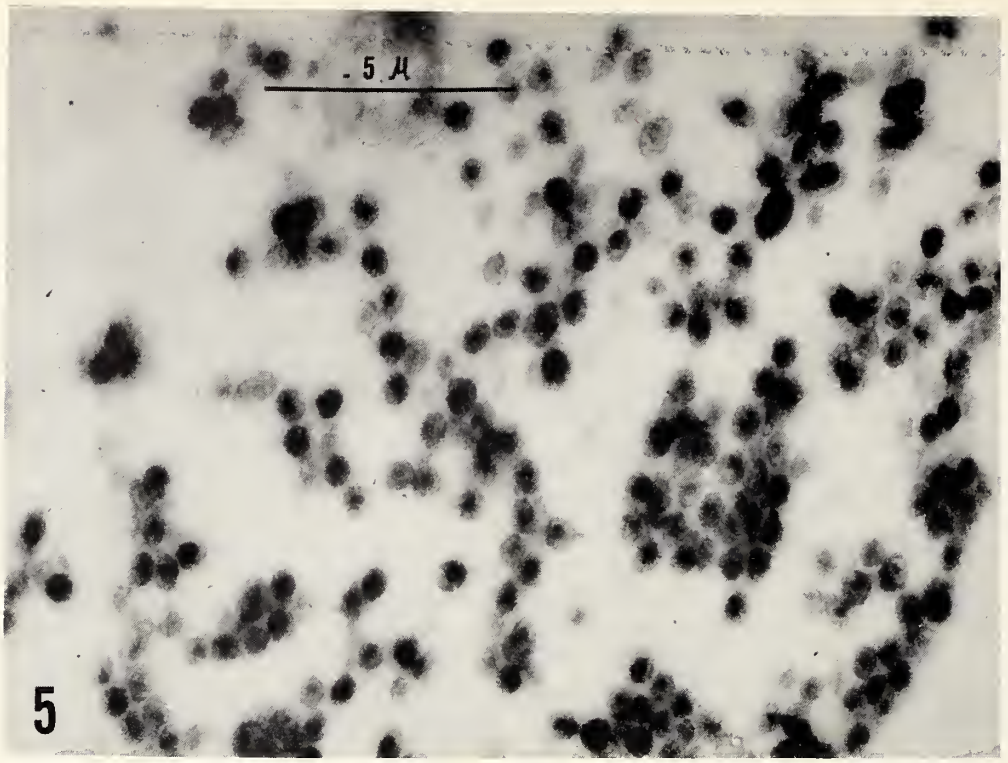


Figure 3.--Complete virus particles containing a central electron dense nucleoid.

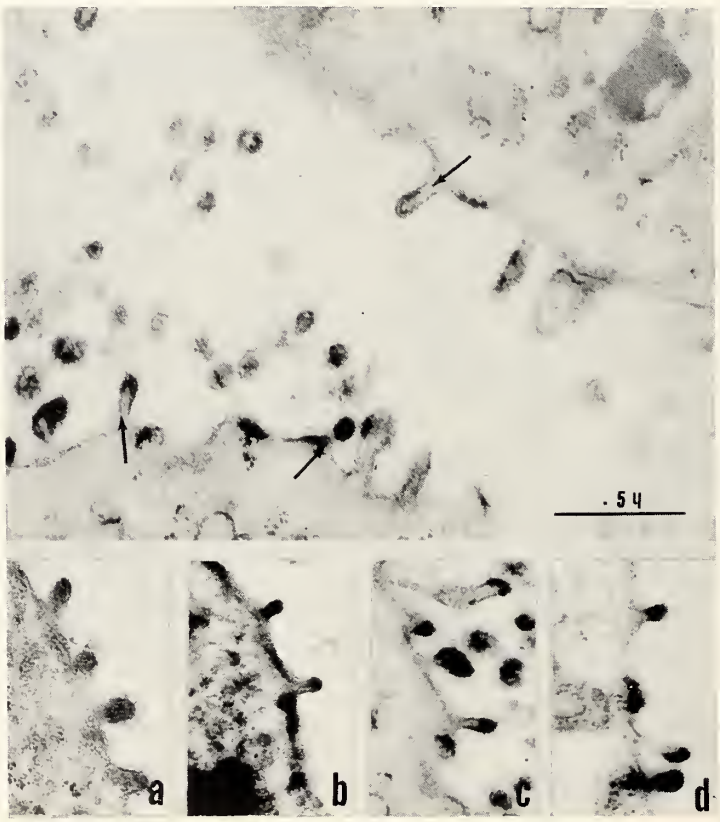


Figure 4.--Various stages in the formation of virus particles at the cell membrane and numerous particles in the intercellular space.

As I have indicated, the lymphoid form, or lymphomatosis, is the most important disease as far as the natural occurrence is concerned. Only sporadically has myeloblastosis and erythroblastosis occurred under natural conditions and these have always been easily transmitted with filtrates.

Only in the last 20 years now have we been able to reproduce the lymphoid disease with filtrates, and this had been primarily due to the use of an inbred stock of chickens which have been developed for susceptibility to this form of the disease.

Now we can go out into the field and obtain tumor material or oral washings or blood or most anything from a diseased chicken or even from a healthy chicken from an infected flock and reproduce this disease.

In Table 4 is given the data of just one of many isolates that have been serial passaged in our line 15-I chickens. Such isolates have been obtained from all sections of the United States, and have caused an overall similar response. The original material was in this case oral washings from a case of visceral lymphomatosis. It was transmitted serially with extracts of lymphomatosis liver and reproduced at a high rate. The experimental period here is 245 days.

The fourth passage was made with one bird that had erythroblastosis and from here on erythroblastosis was reproduced at a high rate and with a short latent period. You will note also it produces sarcomas and endotheliomas and in many cases extensive hemorrhages.

I can not go into the details of showing what has happened here. I can only say that this is partly due to an increase in titer virus and also in part due to a selection of a particular virus type.

Figure 5 illustrates very nicely what happens when we run a titration experiment, that is, we inoculate chickens with serial tenfold dilutions of the virus. At minus four log dose in this experiment, we obtained primary erythroblastosis in less than 100 days time. After a longer time we get visceral lymphomatosis, the disease that occurs naturally.

Another tenfold dilution reduced the proportion that developed erythroblastosis, and there was an actual increase in the proportion that developed lymphomatosis.

Again, another tenfold dilution resulted in only visceral lymphomatosis, the disease that occurs in the field. And it isn't until we get out to a minus nine log dose that an end point is obtained.

This virus preparation comes from a serially passed virus, similar to the one shown in the previous table, where we first start with the bird with lymphomatosis. By serial passage, and selection of proper doners, we have built up the titer, so that it produces

Strain RPL 30 - Serial Passage
Origin - Pierceton

Passage	Inoculum	Percent of Neoplasms Induced							Total Neo. Mort.	Latent Period (days)
		VL	NL	Ost	Ery	Sar	End	Hem		
1	VL oral wash	75	4	0	0	0	4	4	83	149 VL
2	VL Liver	91	0	0	0	0	0	5	91	151 VL
3	VL Liver	88	0	0	4	0	0	0	92	135 VL
4	Ery Liver	0	0	0	80	3	97	57	100	38 E
5	Ery,End-Serum	0	0	0	93	14	79	31	93	27 E
6	Ery,End-Kid	0	0	0	44	39	72	56	100	30 E
7	Ery,End-Serum	0	0	0	75	42	75	29	88	38 E
8	Ery,End-Serum	0	0	0	100	0	100	70	100	20 E

Table 4

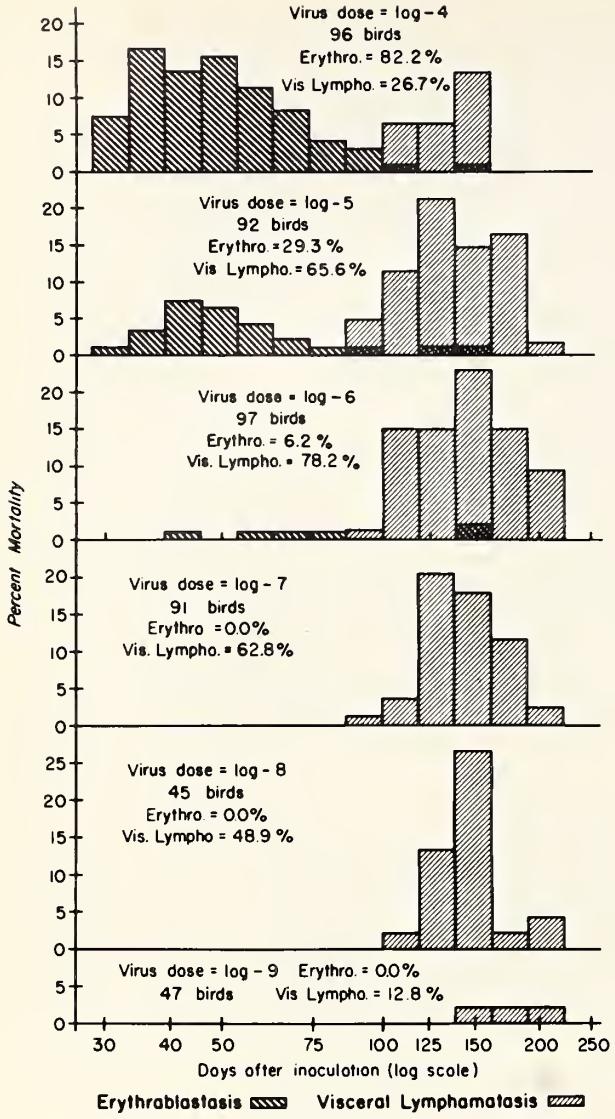


Figure 5.

entirely different types of neoplasms. Then we can recover the field type disease by diluting out the virus and inoculating a low dose.

The results of a contact transmission experiment are shown in Figure 6. Susceptible chickens were inoculated with a high dose of virus, another group was kept in direct contact, and whereas those that were inoculated with high dose develop erythroblastosis as expected, those that were in contact develop only visceral lymphomatosis. The isolated controls remained negative.

This is an ideal experiment. In another experiment, for instance, some of the inoculated chickens might develop visceral and a few of the contacts might develop erythroblastosis.

This again illustrates that the pathologic response is dependent to a large extent on the dose of virus that infects the chicken.

Of course the amount of virus transmitted by contact is small relative to the inoculation dose, and also the route that is useful in natural transmission, we find is much less efficient.

Figure 7 shows the relationship of age at exposure to response of the chicken. A standard virus preparation was used to expose susceptible chickens at different ages. If an intraveneous route is used, a straight line relationship between age at inoculation and the percentage of chickens that develop the disease is obtained. If we use a more natural route, such as nasal inhalation, or oral dosage, a very rapid drop-off at an earlier age is obtained. Even so, older chickens are still susceptible somewhat by this route. Thus, we have here another very important factor which affects response.

In Figure 8, I have portrayed some of these interrelationships, some of them have not been entirely experimentally demonstrated, but they certainly indicate this relationship. A decrease in dose or an increase in age at exposure results in a progressive alteration in the type of lesion from a cytolytic-hemorrhagic to a rapidly growing neoplasm to slow growing neoplasm and hence to a non-lesion inapparent infection.

Now, going further into natural transmission, it was long felt that this disease was transmitted through the egg one way or another. And it was not until we were able to make use of highly susceptible chickens to demonstrate the virus that good evidence was obtained. The only way that we, at that time, could demonstrate the presence of virus was to prepare an inoculum and reproduce the disease in chickens under experimental conditions.

In Figure 9, I have given the results of such infectivity tests. Fourteen day embryos were obtained and inoculum from the liver prepared and groups of fifty chickens were inoculated to determine the presence of the virus.

Three different lines of chickens were selected for testing as to whether they actually shed virus into the embryo. One was a

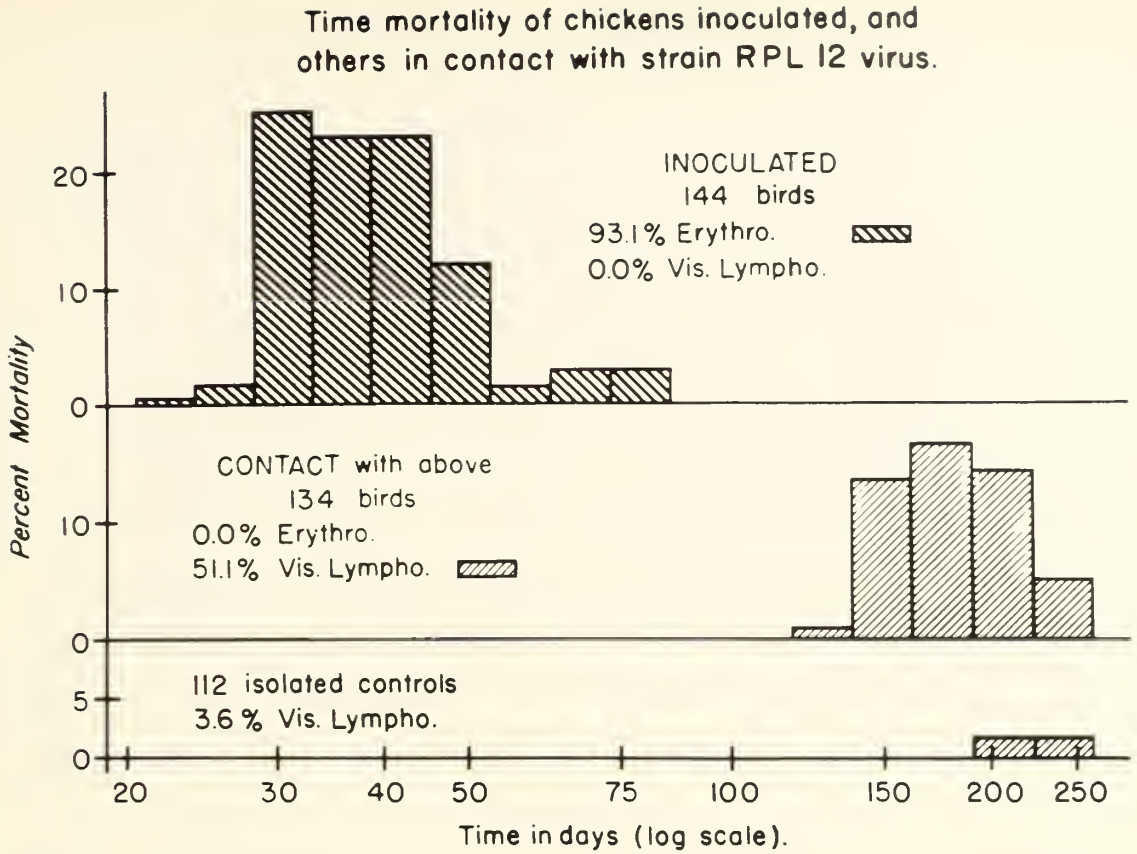


Figure 6.

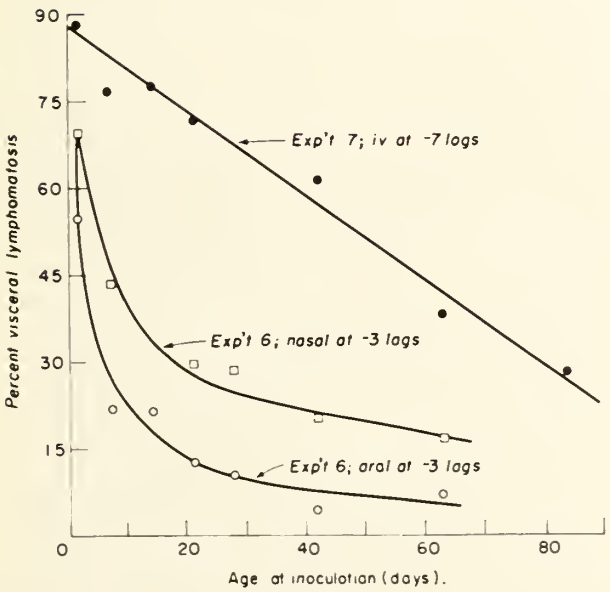


Figure 7.

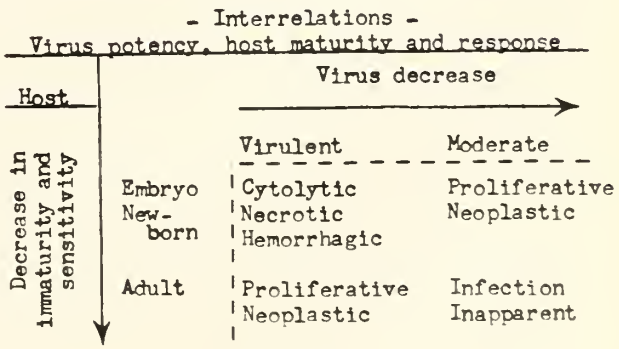


Figure 8.

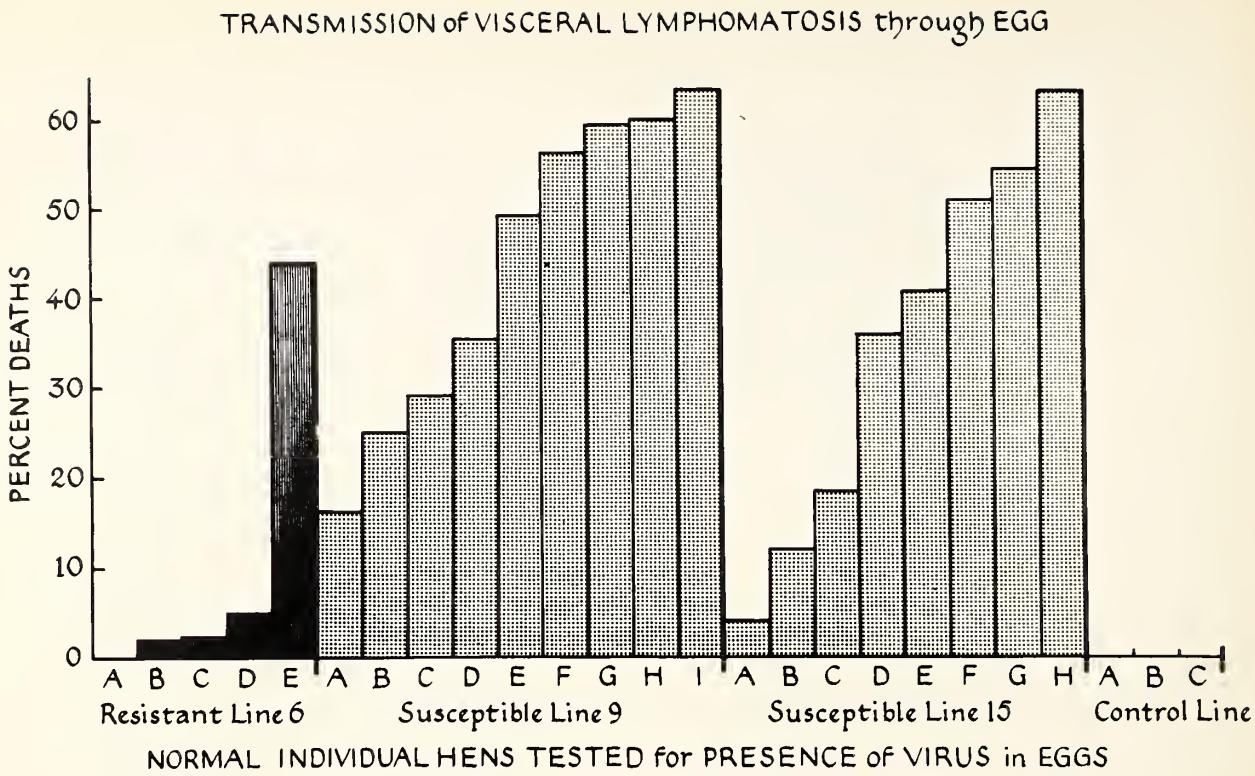

TRANSMISSION of VISCERAL LYMPHOMATOSIS through EGG
PERCENT DEATHS
60
50
40
30
20
10
0
A B C D E A B C D E F G H I A B C D E F G H A B C
Resistant Line 6
Susceptible Line 9
Susceptible Line 15
Control Line
NORMAL INDIVIDUAL HENS TESTED for PRESENCE of VIRUS in EGGS

Figure 9.

resistant line and two were susceptible lines. The height of the columns indicate the amount of transmission obtained with these extracts from normal appearing embryos. If you will note, only one out of five hens tested in our resistant line could be considered to be infected, whereas all of these from this susceptible line and all but one from the second susceptible line, definitely demonstrated that the virus that produces visceral lymphomatosis is present in embryos laid by hens which were perfectly normal and actually laid eggs at a high rate with high fertility.

Table 5 shows the disposition of the dams in the progeny of the hens that we tested. We actually divide them into three categories, i.e., heavy shedders, light shedders, and non shedders.

At the time this table was prepared, we still had many alive that were in this heavy shedder group with only a few with lymphomatosis--actually, 25 percent. There was no mortality from lymphomatosis among the light shedders and none in the non shedders. If we look at the sibs, i.e. brothers and sisters of these hens that were tested, again we get a corresponding relationship; 36 percent in the sibs of heavy shedders against 13 and 0 for the light shedders and the non shedders. The progeny were different. You would expect a much higher correlation here, but it was not really very high. There were cases where many of them were zero, and some of them were very high.

In Figure 10 is data compiled by Dr. Ruben, who developed what is known now as the RIF test. We can now determine or detect the presence of lymphomatosis virus by a tissue culture method which is actually a virus interference test. We determine the presence of lymphomatosis virus indirectly by its ability to inhibit the formation of little tumor foci by the Rous sarcoma virus.

There was a viremic state in some chickens and the progeny of these hens were also viremic.

On the other hand, the progeny from the non-viremic chickens did not develop a viremia. So we have then, two distinct categories in the infected flock, those that are not carriers and are not viremic and a proportion that are carriers, are viremic and have a latent infection. This infection persists; however, the

Table 5.--Relation between shedding of the virus into eggs and the incidence of lymphomatosis in the dams, and in the sibs and progeny

Grouping of dams	Percentage visceral lymphomatosis in test chickens inoculated with embryo liver from dam's progeny	Status of dams	Percentage visceral lymphomatosis in dam's	
			Sibs	Progeny
Heavy shedders	63.5	a	0.0	0.0
	63.4	a	10.0	0.0
	60.2	a	46.0	0.0
	59.5	V.L.	77.0	46.0
	56.5	a	77.0	25.0
	59.6	a	33.0	0.0
	51.1	V.L.	22.0	70.0
	49.4	d	46.0	22.0
	44.2	a	0.0	0.0
	40.9	d	20.0	60.0
	36.1	V.L.	22.0	0.0
	37.5	a	46.0	0.0
Average	51.3		35.6	19.6
Light shedders	29.3	a	46.0	0.0
	25.3	a	0.0	11.0
	18.6	d	11.0	0.0
	16.5	a	8.0	0.0
	12.2	a	0.0	0.0
Average	20.4		13.0	2.2
Non-shedders	5.2	a	0.0	0.0
	4.3	d	0.0	9.0
	2.2	a	0.0	9.0
	2.4	d	0.0	9.0
	0.0	a	0.0	0.0
Average	2.8		0.0	5.5
Total average	33.2		23.2	12.4

a = Still alive.
V.L. = Dead of visceral lymphomatosis.
d = Dead of other causes.

proportion of this type was very small under usual conditions.

As one would expect, the incubation and hatching of infected eggs provide a good source of infection and results in a spread of infection. We have demonstrated--and others have also--that eggs from an isolated clean flock that are incubated and hatched in an incubator, with eggs of an infected flock results in distinct and definite cross infection.

We have demonstrated the virus in the debris of the hatching trays, and there is no question that infection does take place there.

The spread of infection, however, is not rapid and absolute or 100 percent. It depends on what I prefer to call the exposure dose.

Figure 11 illustrates the results of an experiment to show the relationship between the exposure dose and the amount of disease in chickens which are exposed.

In this case, the exposure dose was determined by the percentage of birds that were actually infected.

When there were only 20 percent of the chickens inoculated, the response or the amount of disease in the contact birds was also low. When 60 percent of the birds were inoculated the mortality in the contacts was up to around 60 percent. There was a straight-line relationship between the exposure dose and the response in the experiment.

Another factor is the age of the chicken or length of exposure. Table 6 is an illustration of many experiments showing that when they are in contact from one day on, a high percentage of lymphomatosis mortality was obtained; when the chicks were isolated for the first 30 days, only a moderate percent; when they were isolated for the first 60 days, only a low percent; and when isolated the entire time, 2 percent.

Again, showing the amount or length of exposure is important in determining the extent of transmission.

Figure 12 shows the spread of infection under natural conditions as indicated by the amount of antibody or the number of birds that have antibodies. You will notice that it increases gradually with age. Even though these chickens were exposed in the incubator and constantly exposed during the hatching period, there was only a gradual increase of infection as indicated by the increase in antibody.

I have indicated that the saliva or oral washings are infectious and data in Table 7 demonstrates this infectivity. Again, these figures were obtained by inoculating susceptible chicks with these preparations. These data were of birds that have neoplasms.

As shown in Table 8, similar results were obtained with birds having inapparent infection. Saliva of birds with visceral lymphomatosis gave us good transmission; the oral washings of birds with Rous-type fibrosarcoma were also infectious.

Saliva of chickens infected with strain "A" virus which produces primarily myeloblastosis also produced visceral lymphomatosis.

Infectious material was also obtained from fecal extracts: Results were more erratic, but fecal extracts were definitely infectious.

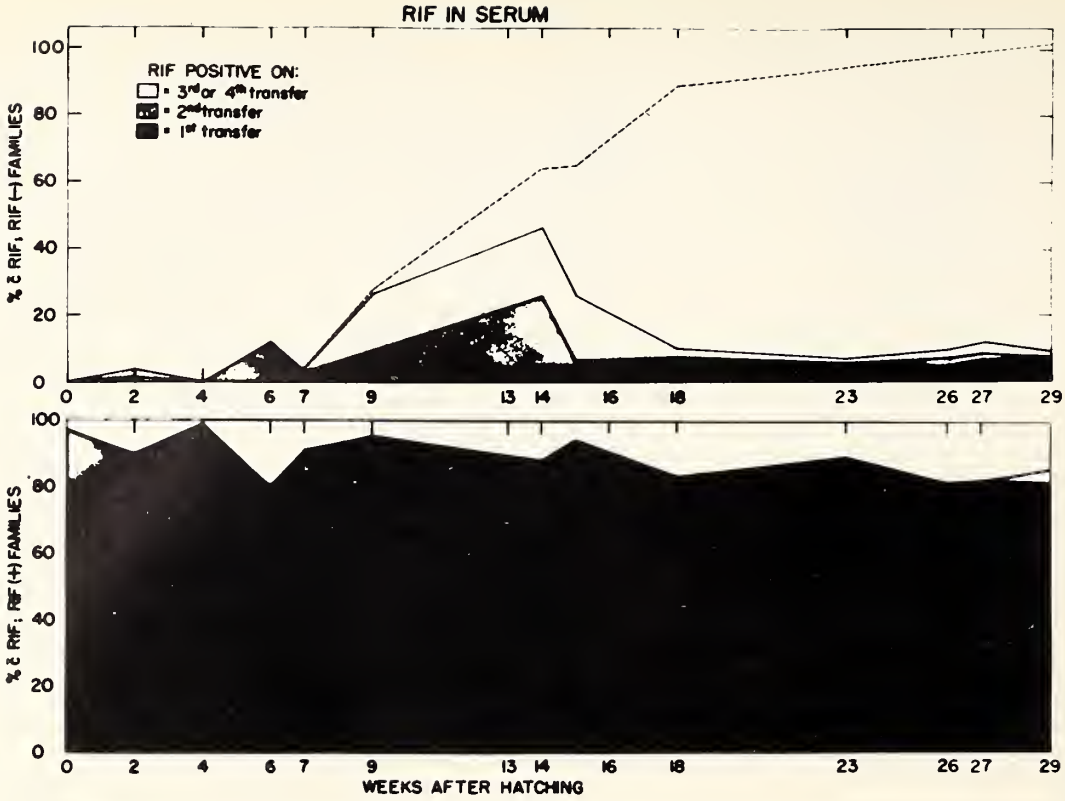


Figure 10.

(from Rubin H., Fanshier L., Cornelius A., Hughes W. F., Virology 17; 143-156 (1962)

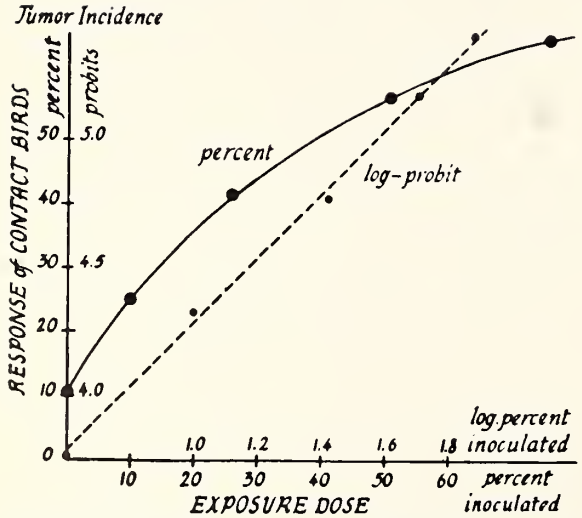


Figure 11.--Relationship between the proportion of chickens inoculated and the incidence of visceral lymphomatosis among the contact chickens.

Table 6.--Effect of Cubicle Isolation for Various Periods on Transmission of Visceral Lymphomatosis in 250 days

Treatment	No. birds	Percent with visc. tumors	Avg. age at death (days)
Inoculated.....	129	48	104
Contact with inoc. birds entire period.....	57	70	180
Cubicle isol. 30 days, then contact.....	50	57	205
Cubicle isol. 60 days, then contact.....	29	21	189
Isolated pen entire time...	51	?	155

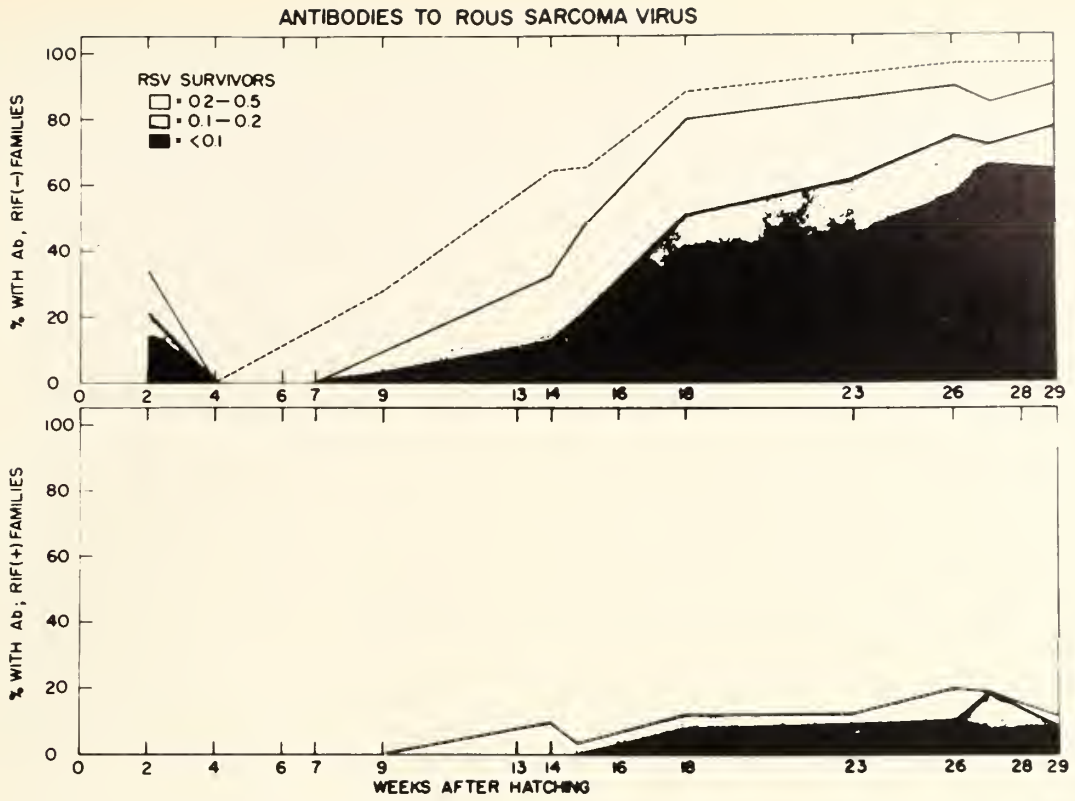


Figure 12. (from Rubin et al. See Fig. 10 VET, J)

Donor chickens			Expt. Number	Infectivity of oral washings: Percent with neoplasms: Vis. lympho.	Others*	Total
With neoplasms						
Contact exposed	lympho.	Pool	62	66	8	74
		Chic. A	38	42	0	42
		" L	41	37	0	37
		" N	39	46	0	46
		" P	36	53	0	28
	Rous fibrosarcoma		49	0	94	94
Inoculated w/ virus	Strain RPL12-Pool		62	65	0	65
	Strain A	Chic. 87	35	29	81	100
		" 90	28	36	71	97
		" 93	32	31	63	94
	Strain R	Chic. 35	44	23	72	93
		" 36	41	7	83	90
		" 43	36	44	39	70
	Rous Sar.	PID 12	20	0	45	45
		" 16	20	0	75	75
		" 20	19	0	90	90

* Includes osteopetrosis, erythroblastosis, myeloblastosis, nepnroblastomas, fibrosarcomas

Table 7.--Occurrence of avian tumor viruses in saliva or oral washings, with neoplasms.

Donor chickens			Expt. Number	Infectivity of oral washings: Percent with neoplasms: Vis. lympho.	Others*	Total
Without neoplasms						
Contact exposed	lympho.	Chic. B	50	12	0	12
		" N	46	15	0	15
		" Q	50	30	0	30
	Strain RPL12	PIO 10	47	0	0	0
		" 30	45	7	2	9
		" 90	48	25	13	35
		" 180	43	35	30	60
	Strain R	Chic. 57	35	29	3	31
		" 58	33	3	21	24
Inoculated w/ virus	Strain RPL12	PIO 10	46	52	2	54
		" 30	46	54	19	69
		" 90	45	69	26	85
		" 180	46	52	47	92

* Includes osteopetrosis, erythroblastosis

Table 8.--Occurrence of avian tumor viruses in saliva or oral washings, without neoplasms.

The results of an experiment given in Table 9 indicate the routes that are actually infectious. High titer propagated virus was administered by several routes with the following results: the eye, 47 percent; mouth 45; air as an aresol 39; esophagus 7; cloaca 56; nostril 73; and trachea 83 percent. Transmission by the esophageal route was low because the virus was placed directly in the crop. It then passed directly to the proventriculous, where the acid probably inactivated the virus before it had a chance to infect the chicken.

In Table 10 are results of our attempts to define more specifically how natural infection takes place. We used as our model the direct contact exposure of susceptible chickens, not previously infected, to inoculate chickens resulting in erythroblastosis. In a previous slide, contact transmission of visceral lymphomatosis was 58 percent. We thought that since the saliva and droppings were infectious we ought to get transmission by this means. But so far we have been unsuccessful.

If we allow them to drink at a common watering trough, i.e., the inoculated chickens were made to drink the same water as the uninoculated chickens, we obtained a good level of transmission. If we transferred the air directly from inoculated to the experimental, again, we were unable to get transmission and finally when droppings were transferred daily from inoculated to the experimental group evidence of transmission was not obtained.

Table 9.--Relative rates of transmission of visceral lymphomatosis virus via various routes

Item	Percent death with neoplasms
Intra-abdominal.....	82.0
Tracheal.....	83.3
Nostril.....	73.3
Cloacal.....	57.0
Ocular.....	47.5
Oral.....	45.0
Airogenic.....	39.2
Controls.....	2.6

Table 10.--Source of exposure in contact transmission of strain RPL 12 leukosis virus

Treatment	Eryth	VL
Inoculation.....	89	0
Direct contact.....	0	58
Exposure through:		
Water only.....	0	6
Air only.....	0	3
Droppings.....	0	4

The only situation where we have been able to obtain good transmission, at least with the viruses that I am now talking about, is where there has been direct bird-to-bird contact. This is true not only with this strain, RPL-12, but also with Rous sarcoma, the strain A, and strain R viruses.

I should say at this point that there is another virus that is being studied more recently, which is apparently much more contagious and spreads through the air and indirect contact transmission is common. This is a recent finding and we have not had time to accumulate much information on it.

This again emphasizes the variations in the biological characteristics between different strains of the virus that produce similar types of lesions.

I would like to go on to another important area, and that is, variations in the sensitivity to the development of tumors and infection. Most chickens that are exposed to the virus become infected but only small portions of them ever develop the neoplasm. Thus, the latent infection, or the inapparent infection stage of this disease complex, is the most important aspect. There are many factors which have a marked influence on actual tumor development in infected chickens.

Three of the best known, yet they may not be the most important, are genetic constitution, virus stimulated immune response, and physiological changes related to aging.

As I have already indicated, the age of the chicken is very important. There are other influencing factors such as hormones, organ function as yet of an undetermined type, perhaps other disease agents, and even dietary substances.

On some of these, the influence is very small and still not definite.

Many geneticists, over a long period, and Dr. Hutt is one of the most noted, have developed resistant and susceptible lines of chickens. At the same time, these are not 100 percent resistant and susceptible, and

there is considerable variation, especially year to year in the percent mortality that has been obtained.

In Table 11 is shown the variation between lines in the chickens. Yes, these are the inbred lines of the U.S. Regional Poultry Laboratory, and this is the mortality due to lymphomatosis after natural exposure.

In the '61 and '62 population, you will note quite a bit of variation between lines, which is genetic, and also there is a variation between years.

This variation between years, we believe, is almost entirely due to variation in exposure, which, of course, is difficult to control under natural situations.

Lately, we have done a considerable amount of work with inoculation of specific strains of virus, and in the next table, data are given for seven different genotypes inoculated with four different strains.

Table 12 illustrates data obtained for line 6, line 7, line 15-I, and reciprocal crosses for most of them. These chickens were inoculated with strain RPL-12 which produces erythroblastosis and lymphomatosis, depending on the dose of virus, strain A virus, which produces primarily granuloblastosis, strain R virus, which produces only erythroblastosis, and the fibrosarcoma strain-Rous virus.

First lets look at data of the pure line: Line 6 is very resistant to RPL-12 and strain A and susceptible to strain R and Rous virus.

Line 7 is very resistant to RPL-12 and to Rous sarcoma virus, and only moderately resistant to strain A and strain R.

It is apparent that line 15-I is almost completely susceptible to all four of these viruses.

The progenies of line 7 crossed with line 6 and line 7 crossed with line 15 gave moderate susceptibility to RPL-12 and high susceptibility to sarcomas. The reciprocal cross, line 6 x 7, also gave high susceptibility.

I might say again that line 7, though very resistant to this strain RPL-12 virus and to Rous sarcoma virus, has given indication of high susceptibility to the new type of virus which we believe is related more nearly to Marek's disease.

We have here illustrations of marked variations in susceptibility of the different lines of chickens. The virus used for exposure is of course, important in an attempt to develop resistant lines or strains of chickens.

This independent variation of various lines and crosses to the different strains of virus strongly suggests that genetics determined susceptibility to the various viruses, even though the different viruses causing the same neoplasms, are controlled by different genes.

Studies on the mode of inheritance of resistance and susceptibility to two of these viruses has shown that a single pair of genes would account for the results obtained.

We have indications that this resistance, at least for these two strains, operates at a cellular level, i.e., the whole chicken is not required. We can get the same kind of results whether we inoculate the hatched chick, the embryo, or the embryo cells in tissue culture.

I should also say a word with regard to the relationship in response between natural exposure and inoculation. We can easily see that whether or not a good correlation is obtained between natural exposure results and inoculation results will depend on the particular viruses involved.

Years ago, many investigators used a cell suspension, and they obtained a transplantation

Table 11.--Lymphomatosis mortality to 500 days of age natural exposure

Year Hatched	RPRL inbred lines						
	6	7	9	10	14	15	151
	Percent						
1961.....	<1	3	12	12	3	7	27
1962.....	2	14	26	19	20	23	--

Table 12.--Response of 7 genotypes to inoculation with 4 strains of virus (females only)

Chicken Genotypes ♂ x ♀	Erythro.-(lympho.) RPL 12	Virus strains granulo-blastotis St. A.	Erythro-blastosis St. R	Fibro-sarcoma Rous
	Percent with neoplasms			
6 x 6...	26	8	91	100
6 x 7...	24	4	96	100
7 x 6...	14	9	88	100
7 x 7...	0	49	56	7
151 x 6...	85	23	83	100
151 x 7...	42	24	76	70
151 x 151.	91	68	100	96

result. This, of course, has no relationship to the virus induced neoplasm.

Other factors are going to influence the response such as age at inoculation and induced immunity.

Chickens from immune hens have maternal antibody for the first two or three weeks. And then upon exposure they reacquire antibodies but it takes quite a while for a high percentage of chickens to develop antibody.

Figure 13 shows a result of an experiment in artificial induction of antibody. Chicks were obtained from chickens that have been reared in isolation with no previous exposure. Progeny were again obtained after the hens had received immunizing injections. The progeny chicks were exposed to virus. The data show that chicks obtained after the hens were immunized were much more resistant to challenge inoculations at one day of age than were chicks obtained from the same hens before they were immunized.

I should emphasize that this challenge was done at one day of age, which actually measures the amount of passive immunity in the day-old chicks.

Data in Table 13 are the neutralizing indices of the serums of one-day, 10-day, 20 and 40-day chicks from immunized hens and control hens. There is quite a high level of antibodies in the day-old chicks; less in the 10-day--and none in the 20 and 40-day chicks. This is exactly in conformity with what was expected on the basis of other diseases.

Figure 14 illustrates the results of another experiment where day-old chicks of a susceptible source were injected with immune serum and then challenged with virus. Others were injected with normal serum or no serum. The results again demonstrated good protection by the antiserum.

I should emphasize that the antibody is of value only in acting against the virus and has little if any effect against the tumor itself. This is illustrated in the results obtained in our attempts to develop a formalized vaccine. Antibody levels of all birds were determined and then they were classified into groups with neutralizing indices of 4, 3, to 4, 2 to 3, and negative, as shown in Table 14. Of those chickens that died of visceral lymphomatosis, six out of 18 were in the high antibody category and only four were negative. Whereas, of those that were still alive: six out of 23 negative, and there were some with a low level antibody titer. Thus, high antibody levels will not protect chickens against the development of the tumor when antibody developed after infection has occurred.

Data in Table 15 illustrate the action of another factor which has a marked effect on tumor development. These results are an experiment on influence of the principal lymphoid tissues, outside of the visceral organs, on tumor development. These organs are the bursa of Fabricus and the thymus. The influ-

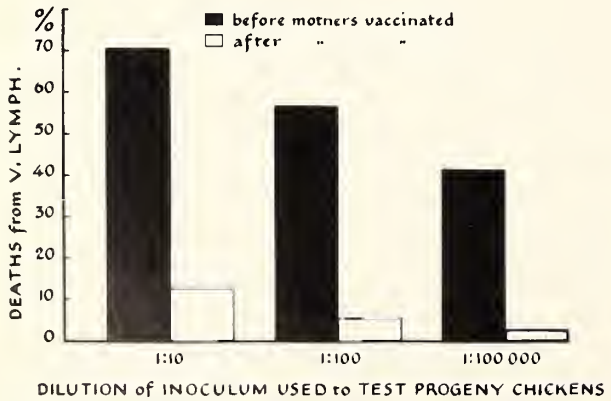


Figure 13.--Resistance of progeny to visceral lymphomatosis before and after their mothers were vaccinated.

Table 13.--Neutralizing activity of serum in relation to age of progeny

Age of progeny	Neutralizing index of serums from progeny of	
	Immunized hens	Control hens
1 day.....	4.5	0
10 day.....	3.3	0
20 day.....	0.5	0
40 day.....	0	0.5

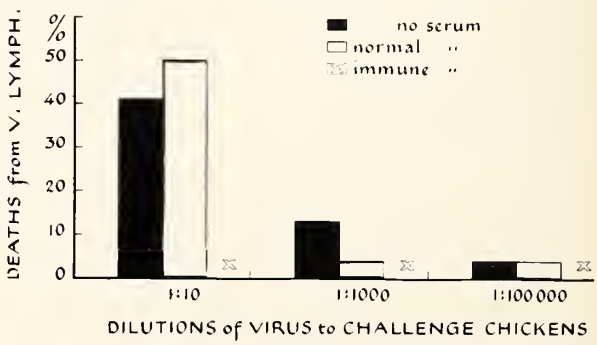


Figure 14.--Protection of chickens from visceral lymphomatosis by injections of immune serum.

ence of surgical removal of both organs and individual organs were studied. The results are on the basis of a 270-day experimental period. The data clearly show that the removal of the bursa of Fabricus at two days or at 29 days of age prevents the development of visceral lymphomatosis while thymectomy has no effect on this neoplasm. Removal of either or both organs has no effect of erythroblastosis or of osteopetrosis.

This is only a preliminary experiment, but certainly looks very interesting from the standpoint of providing conditions or eliminating conditions for the development of the lymphoid tumors.

Table 14.--Mortality in relation to RSV antibody levels (2, 3, 4% formalinized RPL 29-270 PID -4)

Disposition	No. of chickens with N.I.				
	Total	4	3-4	2-3	Neg.
Vis. lympho.....	18	6	4	4	4
Erythro.....	0				
Other deaths.....	5	1			4
Alive to 270 PID.....	23	3	7	7	6
Total.....	46	10	11	11	14

N.I. = Neutralization Index.

Table 15.--Influence of bursectomy and/or thymectomy on the incidence of visceral lymphomatosis in chickens inoculated with RPL 12 L31

Treatment	Group no.	Exper. chicks[1]	Per Cent with visceral lymphomatosis based upon				
			No. dead of visceral lymphomatosis	No. exper. chicks	No. visceral lymphomatosis + survivors to 200 PID	No. visceral lymphomatosis + survivors to 270 PID	Median age at death
2 day operated - 1-day inoculated							
Unmanipulated controls.....	1	41	16	39	76	84	171
Thymectomy and bursectomy.....	2	27	0	0	0	0	--
Thymectomy.....	3	28	8	29	62	81	164
Sham-operated.....	4	36	16	44	89	94	196
Bursectomy.....	5	41	3	7	27	49	--
2 day operated - 28-day inoculated							
Thymectomy and bursectomy.....	6	19	5	16	16	37	--
Sham-operated.....	7	51	34	65	63	65	161
29 day operated - 1-day inoculated							
Thymectomy and bursectomy.....	8	36	0	0	0	0	--
Thymectomy.....	9	38	11	29	85	85	148
Bursectomy.....	10	37	1	3	8	11	--
Sham-operated.....	11	42	12	31	80	92	152

[1] Original number of chickens minus these dying of non-neoplastic causes.
[2] Calculated on greater than 3 deaths.

DISCUSSION

Dr. Morris:

What is the effect on the embryo development of natural egg infection?

Dr. Burmester:

As far as we can tell it has no effect whatsoever.

Dr. Morris:

The chickens that derive from these eggs develop antibodies from the agents?

Dr. Burmester:

These chickens that are infected as embryos are tolerant to the virus since they are infected as embryos, they never develop antibodies or very little.

Dr. Morris:

Are any antibodies of this agent found in man?

Dr. Burmester:

So far we have not found any.

Mr. Yohe:

You made the statement that the difference in the amount of leukosis for the years, was due to the difference in exposure.

Did you mean for that particular experiment or do you mean that in general? For instance, it so happens in Wisconsin, this winter, we have had a rather extensive outbreak of leukosis. Does this mean we have had a difference of exposure?

The second question is: You mentioned about the various factors that may cause leukosis to show up or not show up, does this mean that the virus is there in latent form and certain stress factors bring it out or does it disappear?

Dr. Burmester:

Well, certainly, the exposure or level of exposure is important. We feel that this is, of course, not the only factor.

At the same time, we have information which indicates that it is not entirely the proportion of birds that have antibody or the level of antibody, so we are still looking for other factors. We feel that there are other factors, but these have not as yet been identified.

There have been many suggestions, but none have been proven experimentally. Certainly, the exposure dose is important; if you don't have it, you are not going to get the disease.

One of the big enigmas of this disease complex is why we don't get more disease with the infection that is so widespread. And on the other hand, under certain conditions, we do get high incidence, and we don't have the answer for this.

Mr. Yohe:

The second part of the question was: The organism in cases where you mention such things as diet or other stress factors, does this mean that you have the organism in the bird for perhaps his whole life lying dormant and only showing up under stress factors or does it disappear and determine early whether whether or not these will show up?

Dr. Burmester:

We actually do not know at this time.

We do know that most infections do take place early, that chickens under experimental conditions can have a high virus content for long periods, prior to their development of the disease.

At the same time, as I indicated earlier, there is another type of virus that is running around. We don't know too much about it, but we feel that it is less dependent on early age infection than the more usual visceral lymphomatosis virus.

Dr. Mayall:

The chicken that is infected and carrying the virus, have you ever challenged these chickens with other avian viruses to see if this apparent infection interferes with the development of the challenged infection?

Dr. Burmester:

This goes into the subject of stress perhaps. Is that what you had in mind or was it interference?

Dr. Mayall:

I was thinking more in terms of interference, if you like.

Dr. Burmester:

Again, we have no good evidence for or against this, and all I can do is speculate and I believe you could do that as much as I could. We have used live virus, Newcastle vaccines in conjunction with controlled infection to either interfere or to precipitate disease, but at least, under the conditions of the experiment, we obtained no effect.

Dr. Mayall:

You haven't tried to protect against an infection with say, virulent Newcastle disease virus, that type of experiment?

Dr. Burmester:

No.

Dr. Dickinson:

I found your table where you had the four strains and your 3 x 3 cross most interesting, where you showed interaction between some host strains and some strains of agent. You had these percentages down on your table--could you give me a rough indication of how many animals were in these groups? I was assuming that there was a fairly large number.

Dr. Burmester:

They would run between, say, 25 and 100, something like that.

Dr. Dickinson:

The relatively small differences are reasonably accurately estimated then?

Dr. Burmester:

Yes.

Dr. Stamp:

Could you tell me again about the formaldehyde.

Dr. Burmester:

The data were from an experiment in attempts to develop a killed virus vaccine. We used different levels of formaldehyde and we found that actually it requires quite a bit higher levels of virus and so obtained groups of chickens that received partially killed virus and groups that received only killed virus. We determined the antibody levels of the different groups of chickens and also mortality and came up with the result that you saw.

Paper No. 23

SCRAPIE, A REVIEW OF THE PROBLEM

J. T. Stamp [1]

HISTORICAL

The earliest definite record that has been found of the occurrence of scrapie in sheep in England was that of 1732 (Comber 1772 quoted by M'Gowan 1914). In 1755 it must have been very prevalent for a petition was sent to the House of Commons pointing to the seriousness of the disease and the economic losses which it was causing. During the eighteenth century scrapie was well recognised and freely talked about in many parts of England for the symptoms were often described and the nature of the disease frequently discussed both at agricultural meetings and in the agricultural press. It seems then to have been generally known that scrapie affected many of the breeds of sheep in the south of England, i.e. Norfolks, Hampshires, Dorsets, Wiltshires etc., and also a goodly number of flocks further north. That the disease was indeed a serious menace to many flocks was well publicised for one

[1]Director, Animal Diseases Research Association, Moredun Institute, Edinburgh, Scotland.

can still read many statements made at that time, e.g.:-

> "This disorder has been known to be fatal to the greatest part of a flock and is considered as the most calamitous circumstance the sheepowners have to dread."
> (Claridge, 1795)
>
> "This malady is one of the most fatal that can happen in a flock." (Willich, 1802)

and many more of a similar tenor.

During most of the nineteenth century scrapie either became less common or was less freely talked about; it was no longer of topical interest in English farming circles. Towards the end of last century however the disease must once more have become serious, this time in the flocks of the Scottish Border area, particularly in Northumberland and Roxburghshire; at that time frequent reference to it was made by Border writers and to-day conversation with older shepherds from these areas leaves one in no doubt. The breeds involved were Leicesters, Border Leicesters, Cheviots and the half-breds of these breeds.

Again, scrapie has been well recognised in other European countries--Spain, Germany and France having had the disease for at least as long as Britain if not longer. It has been suggested that scrapie originated in many countries with the importation of Merino sheep from Spain during the eighteenth century. Greig (1940a) however doubts this since he is not convinced that the disease was not endemic in these countries before the arrival of the Merinos. In Iceland a very similar condition called Rida has been recognised for many years.

INCIDENCE IN BRITAIN

To-day the incidence of scrapie in Britain varies considerably from one flock to another. In many affected flocks the number of cases may be only one, two or three a year. This is often the position in commercial flocks selling cross-bred lambs for slaughter where scrapie not uncommonly occurs in the breeding stock and especially in purchased pedigree animals. Since in such flocks both the male and female lambs are generally sold off for slaughter the disease tends to be self-limiting. Numbers of cases occur also in some hill flocks. In other areas, and especially where pure-bred flocks of sheep are kept, the incidence of scrapie may be considerable (up to 50 per cent of a flock) and on some farms the number of sheep lost in any one year may be so large that the loss is disastrous.

Scrapie occurs in a number of breeds of British sheep but it would be invidious to mention any one as being particularly susceptible; the reported freedom of some breeds is often doubtful since the occurrence of the disease is often a closely guarded secret; the shepherd may even conceal the appearance of the disease from his own flockmaster. Suffolk sheep are probably worst affected at the present time however than is any other breed.

AGE OF SHEEP AFFECTED

Field evidence indicates that most cases of scrapie occur in sheep that are between 2 and 4 1/2 years of age but there is good evidence from experimental flocks that older sheep are often affected.

CLINICAL SIGNS

The clinical signs of scrapie can be considered under three headings:

(1) Scraping, rubbing and nibbling of the skin. These are often the earliest signs seen. Loss of wool occurs when the animal rubs itself against fixed objects; the animal bites and nibbles at its skin and rubs constantly with its horns. The "nibbling" reflex consists of the nibbling movement of the lips and licking movements of the tongue when the affected sheep is lightly rubbed over the back, rump and flanks. The animal often exhibits these movements when rubbing itself.

(2) Hyperexcitability, trembling and stupor. Some scrapie-affected animals are hyperexcitable and tremble violently when approached; others are stupid and sleepy.

(3) Incoordination of gait. This is generally more marked in the hindquarters. In the

early stages of the disease the incoordination is most readily observed when the animal is turning or coming to rest after running, stumbling movements then being seen. Later, it shows a swaying movement of the hindquarters and this gradually progresses until the animal is unable to stand. There is no evidence at any time of flaccid paralysis.

Emaciation is frequent towards the end of the disease but a few animals seem to put on weight and become very fat. Occasionally an animal will die quickly after early symptoms have developed.

All animals with scrapie do not necessarily show all the signs. Different features may be present in different animals. The signs of scrapie in one breed of sheep may be quite dissimilar to the signs of scrapie in another breed of sheep, and this is important to emphasize. The clinical symptoms of scrapie in Suffolks are not similar to the clinical signs of scrapie in Cheviots.

EXPERIMENTAL PRODUCTION OF THE DISEASE

Sheep

Until 1936 our knowledge of scrapie was based purely on observation of animals affected with the natural disease but in 1936 Cuillé and Chelle claimed that they had transmitted the condition by injecting brain and spinal cord from a scrapied animal into healthy sheep, the route of infection being intraocular, intracerebral, epidural or subcutaneous. The incubation period was very lengthy (many months or years). This finding was later confirmed by Gordon (1946, 1957) and by Wilson, Anderson and Smith (1950). The latter workers also transmitted the disease in series from sheep to sheep over nine passages. Wilson and his colleagues, using gradocol membranes (410 mμ size) confirmed the earlier finding of Cuillé and Chelle that the transmissible agent could be filtered through virus filters and they interpreted their results to mean that scrapie was due to a virus infection of the central nervous system with an incubation period of eighteen months to five years.

Further experiments carried out by Wilson (1954) indicated that the transmissible agent is unusually resistant and that, amongst other things, it can survive at 100°C. for 30 minutes. Gordon (1946) and Greig (1950) had already shown that the agent resisted a concentration of formalin of 0.35 per cent acting over a period of at least three months. Subsequent studies on the transmissibility of scrapie to sheep carried out by Stamp, Brotherston, Zlotnik, Mackay and Smith (1959) confirmed further and amplified these findings. They found that Cheviot lambs inoculated intracerebrally within 24 hours of birth with scrapie brain developed symptoms of the disease before they were 9 months old, that is at an age when natural scrapie is never seen. It was further found that spleen, lymph gland and cerebrospinal fluid taken from sheep affected with scrapie when inoculated into Cheviot sheep also produced the disease. On the other hand, similar Cheviot sheep inoculated with pooled brains (with and without adjuvant) taken from 20 of their normal companions failed to develop the disease within 12 months of being inoculated.

These workers also successfully passaged the scrapie factor blindly at two-monthly intervals through 7 groups of Cheviot sheep so that it would appear the agent does replicate itself on passage since the theoretical dilution in this experiment was 10^{-18}. A straightforward dilution experiment indicated that in fact the active agent in sheep brain can be diluted out between 10^{-5} and 10^{-9}, a dilution one might expect to obtain with a biologically-active particulate agent. That the agent is particulate is also suggested by the fact that the active agent would not pass through a 27 mμ gradocol membrane. Stamp and his colleagues also found that the scrapie agent will without doubt withstand a temperature of 99.5°C. not only for 30 minutes as found by Wilson but also for 8 hours or more. The fact that the brains from the sheep developing scrapie following the inoculation of boiled material were themselves capable of producing scrapie when injected into further sheep indicates that the action of the boiled material is not simply that of a toxin. More recent experiments at Moredun indicate that serial passage can be achieved by using boiled material at each stage. The active portion of

the boiled material, like that of the unboiled, will not pass a 27 mμ A.P.D. membrane.

Although, as stated above, scrapie can be readily transmitted to sheep it must be emphasized that not all inoculated animals develop the disease (0 to 80 per cent) and that the incubation period varies considerably. It would appear that the breed of sheep inoculated is of considerable importance in determining both the incidence and the incubation period when the agent used is the standard Cheviot one. In this connection it is important to note that at Moredun we have had considerable difficulty in producing scrapie in inoculated Suffolk sheep.

Goats

In 1939 Cuillé and Chelle working in France transmitted scrapie to two goats, the only two inoculated, by the intraocular route. In 1957 Pattison confirmed the French work by successfully transmitting scrapie to goats by intracerebral inoculation of brain tissue taken from a scrapie-affected sheep, while Gordon and Pattison (1957) drew attention to the high degree of susceptibility of the goat as compared with the sheep, one hundred per cent of the goats being affected when brain material is inoculated intracranially. In sheep the percentage "take" varies with the type of sheep inoculated and is rarely anywhere near 100 per cent. Gordon and his colleagues have also shown that scrapie can be passaged serially in the goat and that scrapie goat material can be passed back to sheep, again producing the symptoms of scrapie. The same workers also demonstrated that cerebrospinal fluid, pituitary gland, adrenal gland, spleen, pancreas and liver from a scrapie-affected goat produced scrapie when inoculated intracerebrally into healthy goats but thyroid gland failed to produce the disease. Other work at Compton (1961a, 1961b) suggests that there are different "strains" of the scrapie agent, each producing somewhat different clinical signs in inoculated goats, the drowsy type and the scratching type.

Scrapie occurs in the goat, as in the sheep, following oral dosing with scrapie brain. The agent in goats withstands boiling for considerable periods of time in the same way as does the sheep agent.

Several observations made by Pattison and Millson (1961c) are perhaps a little unexpected in that occasionally goats have developed scrapie following inoculation with tissues from clinically normal goats.

Laboratory Animals

Attempts to transmit scrapie to laboratory animals were unsuccessful for many years although Wilson (1954) showed that the agent could survive in guinea pigs without, however, giving rise to clinical disease. The first clinical manifestation of scrapie in laboratory animals was reported from Compton by Chandler (1961, 1962) who succeeded in transmitting scrapie from goats affected with the drowsy strain of scrapie to mice. Further work at Moredun (Zlotnik and Rennie, 1962, 1963) was successful in that scrapie was transmitted to mice from goats affected with the scratching syndrome of scrapie, from affected Suffolk sheep and from a scrapied Southdown ewe. More recently scrapie has been transmitted from mice to hamsters (Zlotnik, 1963) and from mice to rats (Chandler and Fisher, 1963; Zlotnik (1965).* Preliminary reports of the transmission of scrapie from a Cheviot ram to mice have been made (Morris and Gajdusek (1963).

Since the above experimental evidence suggests that the nature of the causative agent of scrapie is a self-replicating particulate substance which is resistant to boiling it should be possible to purify and concentrate the agent. Mould and Smith at Moredun and Hunter at Compton have developed methods for the extraction of the causative agent of scrapie in both sheep and goats. The purified preparations are biologically active and the conclusion can be drawn that the scrapie agent is particulate. It also appears from this work that the agent from goats has identical physical and chemical characteristics to that from sheep.

PATHOLOGY

No consistent lesions have been demonstrated in any organs outside the central nervous system. In the latter the only gross

*Published since Seminar was held.

change observed is an increase in the amount of the cerebrospinal fluid. Microscopical lesions however are invariably present in all clinical cases of scrapie and are confined mainly to the grey matter of the brain and cord. Pathological changes may be present anywhere in the central nervous system and in the sheep consist of a progressive vacuolation and degeneration of the neurones.

In some advanced cases of scrapie, especially in goats, the intercellular ground substance may be affected by vacuolation either in areas of large-scale neuronal damage or in the rostral parts of the brain even without extensive cellular degeneration (Hadlow, 1960; Pattison, Gordon and Millson, 1959; Zlotnik, 1961). Occasionally in some areas vacuolation of the intercellular ground substance may lead to complete disruption of the normal structure of the brain and may result in foci of softening in which a network of fibres with only a few neurones in its meshes remains (Zlotnik, 1961, 1962).

Astroglial, and in some parts of the brain, microglial proliferation may be prominent in centres of neurone degeneration or necrosis. The increase in size and number of astrocytes is specially evident in the nuclei of the inferior olive and the dorsal motor vagus of the medulla, in the inferior colliculus of the mesencephalon, in the thalamus and mammillary body and in the cerebellum (Bertrand, Carré and Lucam, 1937; Hadlow, 1960; Zlotnik, 1958a, 1961 and 1962; Zlotnik and Katiyar, 1961). Microglia proliferation is often seen in the hippocampus and in the hippocampal gyrus of animals affected with the experimental disease (Zlotnik, 1961).

Myelin changes are not characteristic of the disease but in advanced clinical cases perivascular demyelination is present (Zlotnik, 1958a, 1958b). Limited perivascular lymphocytic cuffings were found in many scrapie brains but in view of finding similar lesions in a great number of brains of large numbers of apparently healthy animals this occurrence has to be disregarded in respect of scrapie (Zlotnik and Rennie, 1957).

A detailed description of the histopathology of scrapie will be given in a later paper by Zlotnik.

ETIOLOGY AND EPIDEMIOLOGY

The etiology of scrapie is still obscure and although the experimental evidence is such as to suggest that the cause is a self-replicating agent or virus which will withstand boiling we have been singularly unsuccessful so far in growing the agent in tissue culture or in demonstrating any serological characteristics. So much so in fact that Mackay, Smith and Stamp (1960) investigated a number of other possible explanations for the results obtained in biological experiments while Pattison and Millson (1961c) consider that the agent of scrapie and the agent causing experimental allergic encephalomyelitis are sufficiently similar to make it worthwhile examining the possibility that similarities in pathogenesis exist. However, neither the Moredun workers nor those at Compton have produced any evidence that the agent is other than a self-replicating substance which will withstand boiling.

Probably one of the most important questions remaining to be answered in the investigation of the pathogenesis of the disease is whether scrapie is an infectious disease and, if so, how does the infection spread? There is of course no evidence that scrapie is a highly contagious disease which spreads rapidly through a population but whether contact transmission at a lower level occurs has been much debated, the evidence for and against mainly being derived from sheep in naturally affected flocks. There is in fact little experimental evidence suggesting that contagion occurs. Greig (1940b) in 1932 exposed a breeding flock of twenty half-bred hoggs and six pregnant Cheviot ewes to pasture contaminated by scrapie-affected sheep. The sheep were purchased from farms on which it was thought that scrapie did not occur. The disease developed between 1936 and 1938 in five of the purchased half-breds (possibly in a further three), in one of the purchased Cheviots and in two of the offspring of the half-breds. By 1936 the flock was reduced by unspecified deaths to twelve half-bred ewes and four Cheviot ewes while nine ewe hoggs had been retained. Greig considered that this indicated scrapie to be an infective disease with a prolonged incubation period and that the causal agent could be transmitted mediately through

the pasture. Gordon (1957) also considered that scrapie can spread by co-habitation at pasture from affected to healthy sheep. He gave as evidence that during a large-scale inoculation experiment on a scrapie-free farm scrapie was confined during the first two-year period to sheep inoculated with tissues from scrapie sheep but that in the second two-year period the disease also occurred in sheep inoculated with broth and normal sheep tissues and in non-inoculated controls. Nine such cases out of 150 sheep were recorded. The age of the affected sheep was not given.

On the other hand, Parry (1960) states that he has no evidence to suggest spread of the disease from affected animals to others of the same generation by contact, coitus or contaminated environment.

Evidence that a flock has been free from scrapie for a number of years must in fact be judged with the utmost caution. This is so simply because many flockmasters are not experienced in identifying scrapie and so do not suspect it unless the symptoms are very characteristic. Further, in our own experimental flocks about 10 per cent of our cases of natural scrapie have been found in animals dying accidentally when between one and two years old and a tentative diagnosis of scrapie depended upon the demonstration of early brain lesions of the disease. No clinical symptoms of scrapie were seen in these sheep.

The difficulties of obtaining sheep known to be from flocks which are certainly free from scrapie has been the main hindrance in starting our contact and other breeding experiments at Moredun which are being carried out in co-operation with the Animal Breeding Research Organisation, Edinburgh. It must be emphasised that scrapie only appears in a fairly low incidence in the sheep population of Britain so that the difficulty is not in obtaining animals which are likely to remain free but in obtaining ones which are certain to remain free if kept in a clean environment. Further, the sheep must be of diverse breeding so as to include some animals which are susceptible if, of course, susceptibility to infection with reference to scrapie is meaningful. It is useless to establish that resistant animals remain free from the disease both in contact and isolation! Experiments taking into account these difficulties are in progress at Moredun and some early results do lend support to the idea that contact transmission does occur in sheep.

Contact infection in goats has been reported by Chelle (1942) who noted a case of scrapie in a goat that had been in close contact with affected sheep. The goat was born in contact with a badly affected flock of sheep and remained therein for twelve months, thereafter it was removed. The first symptoms of scrapie were seen when the goat was two years old and these quickly became quite typical. Chelle also refers to a similar happening in two further goats born in contact with infected flocks of sheep. He considered these findings confirm that scrapie is a contagious disease but that prolonged co-habitation is necessary and that young animals are more susceptible than adult ones. Mackay and Smith (1961) knew of no other reports than these of the natural occurrence of scrapie among goats until they observed the disease in a goat which was being used as an uninoculated control in a biological transmission experiment at Moredun. They were not sure whether scrapie in this goat was of natural occurrence and contracted before the animal came to the Institute six to seven months previously or whether it had arisen from contact at Moredun with scrapie-inoculated goats in the same pen. Pattison and Millson (1960) failed to produce any cases of scrapie in 28 goats kept in contact for periods of 24 to 34 months with goats clinically affected with the disease and Gordon (1959) reports that under experimental conditions in which animals are housed indoors there has been no recorded instance of spread by contact from diseased to healthy animals.

At Moredun we have observed clinical symptoms of scrapie which consisted of biting, nibbling and scratching accompanied by incoordination of movement in four goats of differing breed, the four goats along with one other which has remained unaffected never having been inoculated but having been housed since they were day-old animals with numerous scrapie-affected sheep for 40 months. The clinical diagnosis of scrapie has been confirmed by pathological examination.

In view of the fact that scrapie is not known to occur in goats in this country and the goats

in question came from a goat farm where the disease has not been seen it seems almost certain that the disease arose as a result of intimate contact in the pen with affected sheep and this observation adds considerable weight to the tentative conclusions already discussed regarding contact transmission. This finding emphasises the difficulty in evaluating the results of some transmission experiments especially those where long intervals of time elapse between inoculation and the occurrence of clinical disease. It would seem that such difficulties can only be overcome by strict isolation facilities and/or by limiting the period of observation.

Some further evidence that scrapie is a contagious disease comes from Iceland (Sigurdsson, 1954). The disease, known there as Rida, had been recognised for many years and its presence in certain areas but not in others had become an accepted feature of their sheep husbandry. For various disease control reasons, largely unrelated to scrapie, all sheep in a number of extensive areas (which included all the scrapie districts) were slaughtered and such areas were then kept sheep-free for up to three years. Meanwhile, in other districts source flocks of sheep to be used for restocking these areas were given even closer scrutiny than previously and no scrapie was found in them either before or after the restocking operation. Scrapie reappeared in the restocked areas in some of these sheep.

That scrapie is an infectious disease is not of course universally accepted and many flockmasters and some research workers believe that scrapie is a disease of genetic origin (Bosanquet, Daniel and Parry, 1956; Parry, 1957 and 1960). The latter worker considers that scrapie behaves in a similar manner to a single autosomal recessive gene, this gene being closely linked to some economic character for which the pedigree breeder selects, so giving a high gene frequency in certain breeds of sheep. Parry (1960) confirms the many previous findings of other workers that the disease can be passed from affected to clinically normal sheep by inoculation but does not believe that scrapie, being a genetic disease, is naturally contagious. If a lethal recessive gene is in fact solely responsible for causing a disease then a recognised familial and breed incidence requires no further explanation. When, as in the case of scrapie, it is known that the disease is transmissible by inoculation and there is sufficient evidence to strongly suggest that it is contagious then the evidence supporting the hypothesis of scrapie being due to an intrinsic single gene effect must be very clear-cut indeed and must, in our opinion, depend upon the proof of Mendelian segregation. We do not think that the evidence so far given is sufficiently convincing for us to accept the hypothesis.

One of the simplest precautions in a genetic study is to assess whether the disease is transmitted equally by rams and ewes. Dickinson, Young, Stamp and Renwick (1961) have been testing this point since 1955 and the evidence so far is that rams and ewes do not make equal contributions. The offspring of affected ewes have a high chance of developing scrapie, the offspring of some affected rams have a much lower chance. In fact maternal transmission can account for most of the cases of natural scrapie in those experiments which have been going longest and which have given most information so far. It seems clear from the results to date that in scrapie maternal transmission is present to such a degree that most other sources of variation are obscured and that very carefully designed experiments are necessary in order to determine other sources of variation. This important role assigned to maternal transmission in the natural disease receives strong support from the finding that the disease can be transmitted by experimentally-infected mothers to their uninoculated offspring (Gordon, 1959). Thus we are faced with a major obstacle to any simple genetic explanation of the cause of scrapie.

Maternal transmission does not however alone explain the origin of all cases of scrapie which occur in the field and the information from the United States' eradication programme is very clear on this point. Field evidence from pedigree flocks in Britain does in fact suggest that certain rams do play a part in the transmission of the disease but numerous breeding experiments which are being carried out at Moredun have so far failed to determine under what circumstances this does happen.

On balance the experimental and epidemiological evidence is such as to indicate that

scrapie is an infectious disease. Final proof of this of course depends upon isolating the casual agent and much effort is at present being expended in trying to grow a virus in tissue culture and in concentrating it by physical-chemical methods for serological and electron-microscopical examination. Up to the present time serological work has shown nothing of value.

The control of scrapie on a rational basis is dependent upon a successful outcome of present research programmes but in the meantime the elimination of all family contacts would go far in keeping scrapie in check since the disease has strong familial associations. This does require of course considerable co-operation and complete honesty between breeders. It also calls for accurate diagnosis of all forms of the disease and this in my opinion is dependent upon microscopical examination of the brains of suspected sheep. Since the beginning of this century it has been considered that scrapie is a condition associated with disease of the brain and many workers in France, U.S.A. and in Britain have shown that this is so. Twice during this time however contradictory theories to this have been advanced and in both cases the workers concerned claimed that scrapie was caused by obvious disease of the muscle (M'Gowan, 1914; Bosanquet, Daniel and Parry, 1956; Parry, 1957, 1960). Hulland (1958), however, carrying out careful post-mortem and histological examinations of many affected and nonaffected sheep was unable to correlate muscle lesions with scrapie.

REFERENCES

Bertrand, I., Carré, H. and Lucam, F. (1937) Rec. Med. vet., 113, 586.
Bosanquet, F. D., Daniel, P. M. and Parry, H. B. (1956). Lancet, 271, 737.
Chandler, R. L. (1961) Ibid, i, 1378.
Idem (1962) Ibid, i, 107.
Chandler, R. L. and Fisher, Jacqueline (1963) Ibid, ii, 1165.
Chelle, P. L. (1942) Bull. Acad. vét. Fr., 15, 294.
Claridge, -. (1795) "Letters and Papers on Agriculture to the Society instituted at Bath." 7, 72.
Comber, -. (1772) "Real Improvements in Agriculture."
Cuillé, J. and Chelle, P. L. (1936) C. R. Acad. Sci., Paris, 203, 1552.
Idem (1939 Ibid., 208, 1058.
Dickinson, A. G., Young, G. B., Stamp, J. T. and Renwick, C. C. (1961) Ann. Rpt. Anim. Dis. Res. Assocn., Edinburgh, 1960-61, p. 20.
Gordon, W. S. (1946) Vet. Rec., 58, 516.
Idem (1957) Ibid, 69, 1324.
Idem (1959) Rpt. Spec. Meeting on Scrapie. U.S. Dept. Agric., Agric. Res. Service, p. 1.
Gordon, W. S. and Pattison, I. H. (1957) Vet. Rec., 69, 1444.
Greig, J. R. (1940a) Trans. Highl. agric. Soc., Scotland, 52, 71.
Idem. (1940b) Vet. J., 96, 203.
Idem. (1950) J. Comp. Path., 60, 263.
Hadlow, W. J. (1960) Proc. 63rd Ann. Meet. U.S. Livestock Sanit. Assocn., San Francisco, 1959. p. 300.
Hulland, T. J. (1958) J. Comp. Path., 68, 264.
M'Gowan, J. P. (1914) Bull. Edin. & East of Scot. Coll. Agric.
Mackay, J. M. K. and Smith, W. (1961) Vet. Rec., 73, 394.
Mackay, J. M. K., Smith, W. and Stamp, J. T. (1960) Ibid., 72, 1002.
Morris, J. A. and Gajdusek, D. C. (1963). Nature, 197, 1084.
Parry, H. B. (1957) Vet. Rec., 69, 43.
Idem (1960) Nature, 185, 441.
Pattison, I. H. (1957) Lancet, 272, 104.
Pattison, I. H., Gordon, W. S. and Millson, G. C. (1959). J. Comp. Path., 69, 300.

Pattison, I. H. and Millson, G. C. (1960). Ibid, 70, 182.
Idem (1961a) Ibid, 71, 101.
Idem (1961b) Ibid, 71, 171.
Idem (1961c) Ibid, 71, 350.
Sigurdsson, B. (1954) Brit. vet. J., 110, 341.
Stamp, J. T., Brotherston, J. G., Zlotnik, I., Mackay, J. M. K. and Smith, W. (1959) J. comp. Path., 69, 268.
Willich, -. (1802) "Domestic Encyclopaedia." 3, 495.
Wilson, D. R. (1954) Reported by Stamp, J. T. et al. (1959) J. comp. Path., 69, 268.
Wilson, D. R., Anderson, Ruth D. and Smith, W. (1950).
Ibid., 60, 267.
Zlotnik, I. (1958a) Ibid., 68, 148.
Idem. (1958b) Ibid., 68, 428.
Idem. (1961) Ibid., 71, 440.
Idem. (1962) Acta. Neuropathologica, Suppl. I, p. 61.
Idem. (1963) Lancet, i i. 1072.
Idem. (1965) J. comp. Path. 75, 147.
Zlotnik, I. and Katiyar, R. D. (1961) Vet. Rec., 73, 543.
Zlotnik, I. and Rennie, J. C. (1957) J. comp. Path., 67, 30.
Idem. (1962) Ibid, 72, 360.
Idem. (1963) Ibid, 73, 150.

DISCUSSION

Dr. Rea:

Dr. Stamp, I wanted to ask you a question about an omission rather than a part of your paper. Have you observed, as Mr. Parry, the so-called "fat" animal with scrapie?

Dr. Stamp:

Yes, identical, I didn't go over the clinical signs in detail because I said the signs which we have observed are in fact similar to those described yesterday by Mr. Parry.

We have seen these fat animals, especially amongst Suffolk sheep, but not confined to Suffolk sheep. I have seen similar signs in mice, which will be described later today. It only goes for some sheep, of course, not all of them.

Question:

Is there any evidence on whether this agent may be transmitted by some sheep parasite?

Dr. Stamp:

No, this is a question which we have given some thought to, and the only suggestion on this idea comes from Iceland. Workers there have definitely postulated that there is a biological transmitter of the agent, and I think personally that it is quite within the bounds of possibility. But so far as I know no work on this has been attempted. I think it would be very nice to do this work and I look forward to the time when someone will start inoculating insect extracts into mice to see if scrapie can be produced--various types of sheep parasites could be used. I think this is a very sensible suggestion and one well worth following.

Dr. Gordon:

May I add to this that on the basis that some of the evidence about the spread of the disease from animal to animal is a little bit controversial. This rests with the fact that those cases in which contact infection appears to have occurred in sheep have always been at pasture. There has not, to my knowledge, been evidence of spread by contact in sheep kept indoors. So there is just the possibility that there might be some biological activity present at pasture but absent indoors which could account for spread of the disease under certain circumstances. One of the agents that has always attracted my thought, but not yet my attention, is the possibility that, since the disease can be so readily produced by the oral route, intestinal parasites or lung worms might convey this agent into sheep at pasture, and

provided the animal is susceptible it may take the disease. This is a possibility and one that wants to be followed up. It would be out of character, Dr. Stamp, to allow this morning to pass without at least a little bit of debate, and one of the points that you mentioned was that rida in Iceland and scrapie were identical.

Dr. Stamp:

No, I said "almost identical."

Dr. Gordon:

All right. The "almost" then covers the point I was going to mention, and that is that rida in Iceland doesn't appear in the scratching form as seen in scrapie in Britain. This would suggest a slight shade of difference in the agent in Iceland and the one in Britain which supports the view that there are perhaps different types of this transmissible agent. As to your statement that offspring of affected ewes have a higher chance of developing scrapie than the offspring of affected rams, my evidence with the experimental disease confirms this. There is, however, a closer association of offspring with mother than with father and maternal transmission may be responsible for at least some of these cases. I have carried out two experiments in this connection. In the first we started with a scrapie free flock of Cheviot ewes and 4 rams. Ten lambs from the inoculated parents developed scrapie, although they had not themselves been inoculated. In the second, three non-inoculated lambs from inoculated parents developed scrapie. For details see figures 6 and 7 in addendum to my third paper. (Paper No. 5)

Dr. Stamp:

Thank you, Dr. Gordon.

On the question of the insect transmission, one point I would like to emphasize this morning is that until quite recently we were dependent upon sheep and goats as our experimental animals. I am sure you appreciate the difficulties of working with sheep and goats as experimental animals. For instance, yesterday I suggested that someone within the United States should inoculate 30 sheep to see if scrapie was present in certain tissues, but the cost was considered to be too great. So the fact that scrapie is now in hamsters, mice and rats opens up the possibilities for research of the kind suggested.

On the second point of rida: I would agree with Dr. Gordon, that we believe there are different strains of scrapie, and rida is one example. Another example is material from the Himalayas, from sheep in an isolated community up in the Himalayan foothills of India. Again the story seemed to be that the disease of these sheep is sufficiently like scrapie to make us consider that it probably is, but the symptoms are somewhat different in that itching is not present.

So far as Dr. Gordon's question regarding maternal transmission is concerned, I think we should leave this until later. Dr. Dickinson will be discussing maternal transmission in a later paper.

Mr. Yohe:

Two questions. The first one is: Were the goats that picked up the disease on pasture with the sheep?

Dr. Stamp:

No. This is a point Dr. Gordon raised. I meant to answer it. The goats were in a pen with the sheep all the time, on bedding. This doesn't mean to say, that parasites were not present.

Mr. Yohe:

The second question: The thing that bothers me a great deal about comparing inoculation with natural spread is that it has been demonstrated in inoculation that your goats are highly susceptible, and yet you have both goats and sheep in Britain with a rather high incidence of scrapie in sheep, and I would think that if natural spread was like inoculation that you would have a tremendous flare-up of scrapie in goats throughout Britain. In your opinion is there a similarity between the susceptibility of sheep under natural conditions and inoculation?

Dr. Stamp:

I find this question very difficult to answer. I would have thought that some sheep are just as susceptible as goats. Not all of them. All goats seem to be highly susceptible; only some sheep are. Again I think this will be dealt with later.

The point about your goats and your thinking that there should be a flare-up; I think it is

true to say, at least in the goat farms that I know in Scotland, the goats are rarely in contact with sheep. It so happens that goats on the whole are kept by ladies, who don't tend to keep sheep.

Mr. Yohe:

In other parts of the world, in the Middle East and India, the goats and sheep do run together. Does scrapie appear in goats in these parts of the world?

Dr. Stamp:

In the Himalayas I know that scrapie has only been reported in sheep and this is where no goats are kept.

Mr. Yohe:

As far as you know there is no scrapie in North Africa or the Middle East?

Dr. Stamp:

No. So far as we know, but I am highly suspicious of these statements. All it means is that scrapie has not been found. Virus abortion for example was a disease of the Border areas of Scotland until we found the cause, and it was then immediately found in California, Montana, and other States and then later in Europe. It was recognized in India, Africa, and perhaps the Far East. So it is often only a question of diagnosis.

Dr. Dickinson:

Could I just modify slightly the wording Dr. Stamp used in reply to Dr. Gordon's question about rida. We don't want to be misunderstood on this issue. It may well be perfectly true that rida as demonstrated in Iceland represents a different strain of scrapie agent, but the fact that we have different clinical symptoms may equally well represent the fact that these symptoms occur in a different breed of sheep. I don't want us to be tied because of Dr. Stamp's statement not including the fact that scrapie in Iceland has a different clinical syndrome is evidence one way or another as to whether there is a different scrapie strain involved there.

Dr. Gordon:

Added to that one might say the histology of the two diseases is similar, although the clinical signs are a bit different.

Dr. Hutt:

Some Canadian investigations show what I think are amazing differences among sheep in the ability to repel the disease, and I always suspected such differences might have a genetic basis that bears on the problem.

Dr. Stamp:

Thank you, I didn't say that Icelandic workers have found a biological transmitter for scrapie. I said they had postulated a biological transmitter. They haven't demonstrated one. I think it was suggested by Palsson and Sigurdsson, that the number of types of sheep parasites that they have in Iceland is so small that it might be well worth looking in Iceland for this type of transmission.

Mr. Parry:

I think Dr. Stamp and I are in very substantial agreement on many things. Perhaps in the hurly-burly yesterday I didn't get all the facts over I wanted to. May I take them seriatim.

We are in agreement with, first of all, the 10 percent of possible cases of scrapie in your flocks of younger sheep, which were dying of other causes and were found to have scrapie. We would agree with this in general because I am now coming to the view that many of these early deaths are potential cases of scrapie. If we have a sheep that gets cast on its back in our flocks, we immediately suspect these are preclinical scrapie, and we have some evidence which is being published. Perhaps I didn't say that yesterday. Hence, we would agree with Dr. Stamp that this is one thing we have noted. I am sure they often die of other causes. This "casting" could account for nearly 10 percent in some flocks.

Dr. Dickinson:

We observed this, too, and we say this is not unlikely due to incoordination or impared efficiency of coordination.

Mr. Parry:

That is the inference, but it is not recognized.

Dr. Dickinson:

There is no point in calling it preclinical scrapie. This is an observation we have noted also in conjunction with the scrapie syndrome.

Mr. Parry.

This is a matter of terminology.

Dr. Stamp:

There is a possibility that this is a preclinical form of scrapie. Is that not correct?

Mr. Parry:

What we call preclinical. It matters not what we call it. On the goat farm that was under observation, from which four of your five goats came, you had it under supervision. I wasn't quite clear how long. Do you know the age at which the parents of your goats had been under observation?

Dr. Stamp:

Yes. The parents of these goats had been under observation for a considerable number of years and no scrapie had occurred in them.

Mr. Parry:

Thanks very much. May I just make one comment on rida. I had a discussion the other day with Dr. Palsson, the Director of Agriculture, and I think his view would be that it is not necessarily contagious in Iceland. He would think it was possibly a breeding problem. I will leave it at that. I have no means of distinguishing between these two situations. I merely wanted the meeting to know that there is a body of opinion in Iceland who would not agree that it is necessarily contagious.

Dr. Stamp:

May I, Mr. Chairman, ask Mr. Parry for the evidence. The evidence that Sigurdsson and Palsson have given has been published. It seems to be fairly substantial evidence, and I think before it is contradicted one should be able to give absolute instances of the breeding policy likely to have brought about scrapie in the sheep in these restocked areas.

Mr. Parry:

All I can say is that the evidence as known to the second Dr. Palsson is not convincing to him. That was his private view.

Dr. Dickinson:

Could I ask a supplementary question? This is important. Did the second Dr. Palsson say what the evidence was that convinced him? Did you ask him what the evidence was?

Mr. Parry:

I merely asked him as a visitor. He described the rida problem in Iceland. You can take it for what it is worth. I did not ask him what the evidence was.

Dr. Dickinson:

Scrapie, as it must interest those who suffer financially from it, must in the long run be solved not in mice or in goats or in terms of induced scrapie but in terms of natural scrapie as it occurs in sheep, and this is where Parry and his associates and we at Moredun have a community of interest. I have criticized him, as you have heard, and he will criticize me, as you will hear, on our evidence, but believe you me many people who are geneticists, seeing us embarking on a problem in animals which takes maybe up to 10 years to disclose their evidence, treat us as strangely patient beings. It needs some patience, this disease, and it is not a lightly tackled job, but at the end of the day we must talk about scrapie naturally occurring in sheep.

FIELD OBSERVATIONS ON SCRAPIE INCIDENCE

G. B. Young,[1] J. T. Stamp,[2] C. C. Renwick,[3] and A. G. Dickinson[4]
(Presented by A. G. Dickinson)

BREED DISTRIBUTION

The well known apparent breed restriction of scrapie suggests a familial disease. Not uncommon in Swaledales and Dalesbreds, natural scrapie is unknown in Blackfaces, and in North America it occurs mainly in Suffolks. Breed isolation could be responsible, but is far from complete, contact occurring between farmers and sheep at farms and markets. The rise and decline of scrapie within breeds is also reminiscent of a genetic defect. In the twenties it apparently increased in Border Leicesters and Cheviots, but reputedly due to heavy culling of relatives of scrapies, it subsequently declined, although still existing at low levels. In the fifties it increased considerably in Suffolks. An infectious disease could, however, exhibit similar epidemiology. Scrapie was common in halfbreds, when Leicesters and Cheviots were affected but more significant, uncommon in greyfaces where the mother is a Blackface. Thus while breed distribution suggests familial relationships, as with most field evidence, little is certain. The transmissible nature of the disease outlined by Stamp (1962), emphasises that any purely genetic hypothesis needs conclusive proof before acceptance.

THE PROBLEM IN SUFFOLKS

In 1954, preliminary talks with Suffolk breeders indicated a widespread belief that scrapie was familial, if not hereditary. Affected rams and ewes were reputed to leave affected offspring. The disease was attributed to inbreeding in leading flocks and spread from these into other flocks. Dispersal sales of affected flocks were heavily blamed. In 1955, in a closer investigation, a sample of 21 breeders with about 1,000 ewes in North Britain were visited by Stamp and Young. Another dozen flocks, mainly affected, have subsequenly been visited at the breeders' request by Renwick and Young.

THE INCIDENCE IN SUFFOLKS

In 1955, scrapie was fairly widespread in Suffolks. Of the sample flocks, four were currently heavily infected and four had been slightly infected, varying from restricted use of a ram subsequently developing scrapie, to a few isolated cases in ewes possibly scattered over several years. Eight flocks were almost certainly free, and five stated they were free, although this was unreliable. Four of the five free flocks subsequently became infected and probably there are only a few flocks left which have remained completely free from scrapie. Some infected flocks have, however, eliminated the disease, at least temporarily, but whether the current heavy culling of scrapie relatives is reducing its incidence in Suffolks generally is unknown.

In a survey dependent on voluntary cooperation by breeders an accurate estimate of incidence is impossible, the unreliable class seriously disturbing any estimate. Around

[1] Animal Breeding Research Organization, Edinburgh, Scotland.
[2] Director, Animal Diseases Research Association, Moredun Institute, Edinburgh, Scotland.
[3] Clinical Investigation Department, Animal Diseases Research Association, Moredun Institute Edinburgh, Scotland.
[4] Animal Breeding Research Organization, Edinburgh, Scotland.

20 cases, however, were seen to occur in 1955 in the 1,000 or so ewes visited, an incidence of about 2 percent. Since the Suffolk ewe population was about 20,000, a very tentative estimate might be around 400 cases a year. About 8 percent of the 5,000 or so replacement ewes fed in annually might eventually take scrapie. Problems of age distribution and incidence are discussed by Dickinson et al. elsewhere in this symposium.

SEVERELY INFECTED FLOCKS

The disease could obviously be serious to individual breeders. For example, Table 1, flock A of just over 200 ewes has 82 scrapies in 7 years - around 11 cases per annum or a ewe incidence of about 5 percent. The replacements being around 50 ewes per year, about 20 percent of each ewe crop became affected. Flock B, of 60 to 70 ewes, had 29 cases in 8 years, again an annual ewe incidence of about 5 percent with about 20 percent of each ewe crop becoming affected. Flock C had 36 cases in 7 years, 35 percent of each ewe crop; flock E lost 15 sheep or 11 percent of each ewe crop in 7 years; and flock D had 36 cases in 9 years, or 26 percent of each ewe crop.

Thus in these five heavily infected flocks, a high proportion of the replacement ewes took scrapie - over periods of 7 to 9 years, around 200 out of approximately 900 ewes reared to at least one lambing became affected. The proportion of ewes in the flock lost each year, however, was substantially smaller around 5 percent - even in the flock of 200 there were only 11 cases or so per annum, and, in the smaller flocks only 2 or 6 cases per year. These losses, however, continued year after year, sometimes being higher and sometimes lower.

The sheep loss, the heavy culling of scrapie relatives, the disorganization of breeding programmes, the difficulty of becoming free again in a heavily infected flock and loss of repute all combine to make scrapie a dreaded disease. While some breeders manage to live with the disease, and with varying success to cull against it, others like flock D abandon the struggle and dispose of their flock, often at a very heavy financial loss.

DIFFICULTIES OF FIELD DATA

The normal difficulties in establishing the mode of inheritance of a congenital abnormality from field pedigrees are greatly increased in scrapie. The smaller numbers of males reared and their subsequent dispersal before scrapie age confines the analysis mainly to females. Flock sizes are generally small and the limited number of rams and often of offspring per ram may prevent contemporary ram comparisons differentiating sire effects from contact infection. The small ewe families, averaging just over one offspring surviving to scrapie age, render repeatability of ewe performance difficult to obtain. Culling of scrapie relatives may destroy evidence of daughter dam association or of twin association. The classes necessary to differentiate genetic from maternal transmission may be lacking. Shepherds diagnosis is often unreliable, particularly in early cases killed for salvage. Inaccurate and missing female pedigrees have caused flocks to be abandoned for our further consideration or rendered conclusions doubtful.

Table 1.--Natural scrapie in five heavily affected Suffolk flocks

Flock	Number of ewes	Number of cases of scrapie observed	Number of years	Scrapie cases per year	Ewe incidence per year	Homebred ewe input into flock per year	Proportion of homebred input affected
A.....	200	82	7	11	5%	50	20%
B.....	60-70	29	8	3-4	5%	15	20%
C.....	55	36	7	5	9%	15	35%
D.....	65	36	9	4	6%	15	26%
E.....	80	15	7	2	2 1/2%	20	11%

Further, culling poses serious difficulties in calculating genetic ratios, preventing many ewes reaching scrapie age, some of which given time, would develop scrapie. Genetic ratios calculated using culled populations would produce a deficiency of scrapies due to misclassification of scrapies as normals. If age incidence is known, corrections may be made for misclassified ewes, by estimating the proportion of ewes culled at different ages, which would take scrapie. Age incidence estimates, however, would be biased if culling for scrapie was not at random, which is unknown, and they also depend on accurate culling rates which are only approximately known. Genetic ratios from culled field populations would therefore be dubious.

It was thus unlikely that clear cut genetic relationships in scrapie were obtainable from pedigrees. The most to be anticipated was the emergence of indications of familial relationships.

PEDIGREE ANALYSIS

Pedigree diagrams of the five severely infected flocks, previously mentioned, were scanned for familial relationships. The C flock used two affected rams siring many scrapies, but there was also a scatter of cases through the flock. In the E flock, an affected ram similarly sired many cases but here also there was a contemporary scatter of cases of unknown origin. In the B flock, scattered cases were the main feature although one apparently normal ram sired many cases. In the D flock, culling of offspring of affected mothers was not practiced and many scrapies were out of scrapie mothers. The origin of many cases was, however, inexplicable. In the well recorded A flock, the offspring of two affected sires, as well as the offspring of affected ewes were culled and practically all subsequent scrapies were out of apparently normal, in many cases old ewes. They occurred at random through the flock, no familial pattern being detectable.

While some pedigrees thus suggested, quite strongly, familial relationships, none could be interpreted easily on genetic grounds alone, due to the scatter of inexplicable cases, often from many different rams, pointing to infection possibilities. Equally, apart from the A flock, it was difficult, although, in the last resort, not impossible to interpret the pedigrees in terms of contact infection alone - the familial relationships were too obvious. These are now examined in greater detail.

SIRE TRANSMISSION

In flock C, an affected sire, I, left 7 affected and 7 normal but no contemporary rams were available for comparison. Subsequently another affected sire, II, left 12 affected and 5 normals in two years while an apparently normal ram, III, used contemporaneously left 16 normals and 2 affected and another normal ram, IV, 9 normals and none affected. Flock E purchased I and he left 5 affected and 12 normals while a normal ram, VI, contemporaneously left 10 normals and none affected and another normal ram IV left 6 normals and one affected. In none of these cases, however, was a comprehensive maternal history available, although none were known to be scrapies. Moreover, both mothers and offspring were of varying ages. The frequency of affected offspring in the 'both parents affected' class (qv.) also suggests the significance of an infected sire effect.

Old apparently normal rams possibly may also leave a high proportion of scrapie. In flock B, ram VII, which lived to an old age and was confirmed as unaffected, was reputed to leave 8 affected and no normals while contemporaneously 4 other rams left 27 normals and 7 affected. Ram VII was also reputed in another flock to leave a high proportion of affected progeny. Again, however, neither adequate maternal history or diagnosis was available. In contrast, in another flock, F, an affected ram used on previously clean ewes has left 8 unaffected offspring, although these are now over 4 years old. A similar experience occurred in a Leicester flock.

Pedigrees thus suggest affected sires, and possibly apparently normal sires, can, although not necessarily always, transmit scrapie or scrapie susceptibility. Very many normal sires can, however, sire scrapie. In flock A, 18 out of 21 apparently normal rams used have sired scrapies. They were generally used for two or three seasons, 7 or so being used more or less

equally each year, scrapies appearing in their offspring year after year. Rams such as IV and VI and even III suggest, however, some rams may leave a large proportion of free offspring even in an affected environment.

DAM TRANSMISSION

Three flocks provide some suggestion of dam transmission. In flock D, 11 out of 33 cases and in flock E, 4 out of the 9 cases, not out of ram I, had affected mothers. In flock B, a grandmother, mother and daughter were affected. Old ewes often, however, leave affected cases, e.g. in flock A, 29 cases were out of 5 year or older mothers.

BOTH PARENTS AFFECTED

Eight offspring from such matings took scrapie and none survived to over 4 years, without taking scrapie, suggesting most such offspring may become affected. Parry (1962) obtains similar results from larger material.

TWINS

While in 10 twin pairs, possibly a high number, both members were affected, and in several pairs one member was affected and the other still free after a year or two, culling of the free twin before 4 years made legitimate comparisons impossible. Several pairs of scrapies occurring in relatively free sections of the flock, however, suggested familial relationships and some occasional triplets did the same.

GENETIC RATIOS

The material was searched for evidence of genetic ratios, indicating a simple recessive. Theoretically if sufficient were known, parents could be classified as homozygous recessive, heterozygous and homozygous normals and genetic ratios in the offspring of classes such as affected x affected, carrier x carrier and free x free could be examined to see if they fitted a recessive. To tentatively establish the status of homozygous normal or heterozygous, animals have to live at least 4 years, have both parents observed for four years and to establish ratios the offspring have also to live for four years. Unfortunately sufficient numbers of such classified animals were unavailable to obtain worthwhile ratios. The flock A, however, with 18 out of 21 rams, two thirds of which were bought in, having scrapies is almost sufficient, by itself, to exclude a recessive. The high incidence in the offspring of rams I, II, and VII also tends to exclude a recessive.

CONCLUSIONS FROM SURVEY

This small survey thus suggested the likelihood of sire heterogeneity with some but not all affected rams leaving a high proportion of scrapies, a mother offspring association, a high incidence from scrapies mating together and the possibility of both members of a twin pair being frequently affected. A simple recessive, therefore, seemed extremely unlikely. While the wide distribution of cases in the different flocks suggested other factors besides the familial one were operating, sufficient evidence of familial patterns existed to justify experimentally investigating these amongst other objectives.

INBREEDING AND SCRAPIE

Many breeders emphasised that scrapie was associated with inbreeding, although there was no evidence of this in the infected flocks, which avoided inbreeding by purchasing rams. Since females of the Suffolk breed are only recently officially pedigreed, the inbreeding in the breed as a whole could not be measured. Border Leicesters, however, are fully pedigreed and apparently managed similarly to Suffolks and, in them also, the scrapie epidemic in the thirties was attributed to inbreeding. The total inbreeding in Border Leicesters (Purser and Young, 1962) was, however, only 5.8 percent, a figure not suggesting excessive inbreeding.

RECESSIVE GENE

Parry (1962) and Draper and Parry (1962) produced field data prima facie suggesting a recessive. The main and a strong case is that

affected x affecteds, leave mainly affecteds. Other explanations are, however, possible, e.g. a combination of ram and dam transmission of an infectious agent or of susceptibility genes. Apart from this the evidence is not strong, the remaining classes, e.g. being based on small numbers or not providing good fits. The excess of scrapies is explained by preferential selection of presumed heterozygotes and homozygous recessives, for which there is no evidence. Parents are classified in many cases on their offspring, a procedure which must be treated with considerable caution and is unobjectionable only if family sizes are large. Otherwise circular arguments result. Reciprocal crosses necessary to differentiate maternal transmission are missing in the classification they use. The class of homozygous normal rams leaving only normals out of affected mothers is absent from the main body of data (Parry, 1962). The pedigrees can be interpreted as due to infection and the data in other ways - e.g. maternal transmission. Indeed, for the data given for twins there is an excess of cases where both are like their mother and a deficiency of pairs where one is affected and the other not, again suggesting the importance of the mother's status. These data, as with ours, indicate familial relationships but do not prove that a recessive gene causes the disease.

CONTROL MEASURES

Some flocks remain relatively free by purchasing stock - if possible - from clean flocks, using only older rams or stock out of older parents, using rams carefully until proven, and keeping the flock self-contained. Why other flocks remain free is inexplicable. Only in slightly and moderately affected flocks, can breeders vigorously cull relatives of scrapies, principally affected sire and ewe progeny groups. In several flocks vigorous early action appears to have restricted the disease spreading. In severely affected flocks, such as flock A, culling can only be partial - some sheep must be left and other characters selected. While culling is frequently useful, e.g. in flocks C and E, both of which following culling are now relatively free, culling has sometimes apparently little value, e.g. in flocks A and B. Flock disposal may be necessary, as in the D flock, where a sire, which subsequently developed scrapie at an old age, and one of its sons were extensively used in an already heavily infected flock. Culling cannot be based on a recessive hypothesis - probably most rams would then have to be culled. While probably controlling scrapie, either genetically or by removing sources of infection, it is unlikely to eliminate it from the breed. Until contact is proven, appropriate advice is impossible and probably mixing scrapie with clean flocks is best avoided. In a population already heavily infected, really effective control can only depend on an accurate knowledge of the aetiology of the disease.

NECESSITY FOR EXPERIMENTS

Such accurate understanding of the disease is unobtainable from field data alone. Experiments, where the disease 'stews' under proper observation, with proper diagnostic facilities, with designed mating and retaining animals to an adequate age are essential. Some results from such experiments are described elsewhere in this symposium.

REFERENCE

Draper, G. J. and Parry, H. B. 1962. Scrapie in sheep. The hereditary component in a high incidence environment. Nature 195, 670.

Parry, H. B. 1962. Scrapie: a transmissable and hereditary disease of sheep. Heredity 17, 75.

Stamp, J. T. 1962. Scrapie: a transmissable disease of sheep. Vet. Rec. 74, 357.

Young, G. B. and Purser, A. F. 1962. Breed structure and genetic analysis of Border Leicester sheep. Anim. Prod. 4, 379.

DISCUSSION

Dr. Carter:

With respect to 5.8 percent inbreeding in the Border Leicester, that was relative to what date?

Dr. Dickinson:

This was postwar. Most of this data was postwar. They worked for a longer period, and I can't remember if the 5.8 is the last period they dealt with or an average.

Dr. Carter:

As I recall, the rate of increase was something on the order of a half percent per generation, and that's been true of almost all the breeds.

Dr. Dickinson:

I simply wanted to quote this figure because it is the average figure for most livestock inbreeding. It is a factor that very often crops up in breeders' minds. This doesn't apply to a single flock where entirely different conditions may apply. I simply wanted to state that on average there is nothing very serious about this. I don't think this could be used to completely knock down the notion that inbreeding somehow or other had some particular relationship to the origin of scrapie. I say this because we are inbreeding Suffolks for this particular reason, but there is no high amount of inbreeding going on en masse.

Mr. Draper:

Could you repeat your figures on progeny where both parents were affected and tell us whether any of this was any of the twin data that is concerned here?

Dr. Dickinson:

I think there was one pair of twins in these eight animals. I only "think" because this part of the work is mainly Dr. Young's. This is his aspect of the data. He has all the diagrams, and since we based no conclusions on these I can only say hesitantly that there were only eight cases. They all died before four years, and concurs entirely with Parry's data on this point and our own experimental data. I think there was only one pair of twins among these.

Dr. Stuart:

What evidence do you have that scrapie has been controlled or eradicated in any of these flocks by using culling methods alone?

Dr. Dickinson:

I can simply point out the fact that the best documented evidence that we have on this for one flock where culling has been so rigorous over the years, from the early 1950's to late 1950's, that the average structure of the flock has changed quite radically; in this particular flock, Flock A, there is a strange distribution of cases. In all the other flocks you get certain hints: you get cases where an odd set of triplets go down; you can get cases where obviously you can treat one part of the flock as being almost free from scrapie and another part where scrapie is occurring, suggesting familial relationships. But the flock that we know has been most systematic in changing its breeding policy apropos of trying to get rid of scrapie is Flock A where the cases are distributed at random. They are here, there, and everywhere, just isolated cases dotted over the years. I can't interpret that beyond saying that it is most strikingly different, and the owner is a man who is most systematically culling.

Whether the culling in fact has produced this difference in apparent distribution or not is a matter for speculation, but obviously all I can give is a simple answer to your question that some flocks which do cull heavily have plenty of scrapie left with them.

Dr. Stuart:

Regarding the ewes that were introduced into these infected flocks, did you have a history of those ewes, and how long after they were introduced did they develop scrapie?

Dr. Dickinson:

We might be speaking at cross purposes here about introduction.

Dr. Stuart:

I may have misunderstood. I was under the impression there were so many cases in which clean ewes were introduced into an infected flock and later developed scrapie.

Dr. Dickinson:

No. There are just largely flocks which are only introducing an occasional ewe relatively

rarely, and the introduction basically is of rams. In Flock A, two-thirds of the rams that he used are introduced rams. One-third are homebred. In that particular flock I think it will be true to say there are no introduced ewes whatsoever. This is not necessarily true of flocks B, C, D, and E. There is certainly in one of those flocks some introduction of ewes, but the introduction is a negligible part of the general story. Introduction of rams is an entirely different question, and we are dealing here with data mainly on ewes and not on rams. These are largely self-contained flocks.

Dr. Stuart:

Is there some evidence that negative animals introduced into infected flocks acquire scrapie by contact?

Dr. Dickinson:

After I have presented my data on experimental breeding of Suffolks, I would be willing to answer you then, since it is an important question, but I think I would much rather present an answer to it in the light of those findings than of these.

The answer here, as I must keep stressing, is you can squeeze this kind of data like mad and it doesn't drop one ounce of blood, in our estimation.

Dr. Gordon:

You have roughly eight offspring from two affected parents, and the statement is that these died before they were four years old. Did they die of scrapie?

Dr. Dickinson:

Yes, they all died of scrapie.

Mr. Parry:

The ewe input is from homebred ewes, isn't it?

Dr. Dickinson:

Yes, but it is not as easy to make this judgement as all that. The Suffolk breeders have developed little whirlpools within the breed structure because of scrapie. You have half a dozen breeders who know and trust each other, and they do exchange some ewes between one another. They do in fact use each other for sources of rams, and they wouldn't trust a whirlpool next door to them, which is again a little clique of breeders who trust each other and tend to try to swap their rams among themselves. There are exceptions to this, but generally this is the picture; there can be some interchange of ewes within the group but mainly of rams.

Mr. Parry:

They can always get much more money for their rams in Scotland than we can in England by this method. I would point out in my data without exception we have no such ram syndicates. We haven't had. We have them, but we will talk to them tomorrow. Do you know the source flocks from which they got their rams about which we are talking?

Dr. Dickinson:

The evidence here with regard to Flock A is that I think he is outside a whirlpool. This is just by pedigree analysis. We have in fact some flocks, our main free flock to which I will be referring later--that is our biggest free flock--which we believe to be free and has remained free for many years. In terms of an analysis of the flock content of rams, it is different from the situation which you find in many of the flocks where you have a heavily affected situation.

Dr. Hutt:

In point of fact, at this stage in the proceeding, you don't know enough about the source flocks from which these sires were obtained to have any real opinion? I appreciate this point.

Dr. Dickinson:

No. I am trying to say that we haven't thought it was worth going on to do this. We have just simply checked up in one or two cases. Flock A we know a lot about, because it puzzles us epidemiologically. The free flock I referred to, we know a lot about, because one wants to know something about this whirlpool situation. But we have only dipped back into that sort of information to answer occasional questions or confirm occasional findings.

Dr. Hutt:

This sort of pattern is one I am very familiar with. The point was when you say a ram is unaffected, you didn't tell us what age.

Dr. Dickinson:

No, I don't pretend for one moment that this means anything in your sense of the word. The animal is normally observed to three and a half years old. But I don't want to build anything one way or the other out of it. I am simply saying in Flock A, those 21 rams, some of them might have been observed as old as five and a half, but relatively few. Most of them were probably observed no longer than four years. But one breeder kept repeating the use of his rams for two or three years. This makes them at least three and a half years old by the time they are disposed of. But I don't want to make any claims on freedom, had they been kept longer.

Dr. Hutt:

The use of unaffected, fair enough, is not the same as my firm definition of unaffected.

Dr. Dickinson:

No. I wouldn't want to claim that whatsoever.

Dr. Hutt:

How many visits does Young or Renwick or Doctor Stamp put into these flocks?

Dr. Dickinson:

Quite a lot of these data are from flock records.

Dr. Stamp:

I could answer that. Farm B, C, D, and E, I should think we must visit five or six times a year.

Dr. Hutt:

Have you any idea or any corresponding data for flocks that had scrapie for some years, but at lower levels and if so, at what levels?

Dr. Dickinson:

You can go through the range to zero over a number of years. Are you trying to ask what proportion of flocks these represent and what proportion would have a percentage of input ewes affected in the order of five percent and what portion of those would be having zero incidence?

Dr. Hutt:

Not quite. If you have zero, of course, you can say the flock has never been exposed. But I mean a flock that has had scrapie for enough years for you to believe that it is there on the premises, but perhaps with lower morbidity rates. I can put it another way. Are there any flocks in which the mortality from scrapie, the expectance, might be on the order of five percent?

Dr. Dickinson:

Oh, yes. But I think we can cite experiences where flocks have remained free for the order of 30 or 40 years. We will say they didn't know it after the first World War, this sort of situation.

THE DISTRIBUTION OF SCRAPIE IN SHEEP OF DIFFERENT AGES

A. G. Dickinson[1], G. B. Young[1], J. T. Stamp[2], and C. C. Renwick[3]
(Presented by A. G. Dickinson)

Is there an age at which scrapie most commonly appears? Is scrapie so infrequent in sheep over a certain age that they can be regarded either as non-susceptible or even as scrapie-free? An examination of these questions will demonstrate some limitations of field evidence in addition to those already discussed.

The half-life of a Suffolk ewe in Britain is about 4 years - by this age there is about a 50 percent chance that any individual ewe will no longer be present in the flock for any of a variety of commercial reasons. In the absence until very recently of detailed records of females in the Suffolk Flock Book, it is not possible to make a broad-based estimate of culling rates at each age in this breed, though it can be shown that one-quarter to one-third of the total females in the Suffolk Breed are replaced each year. This is in accord with the records for the limited number of Suffolk flocks used in our current work. Culling-plus-death rates based on these sources are presented in figure 1.

Therefore for any scrapie animal which is observed at, say, 7 years old approximately four similar animals will have been culled and are therefore not recorded as affected. This assumes that animals prone to develop scrapie are not kept in preference to animals which cannot develop the disease; in other words, culling is uncorrelated with scrapie susceptibility and there is at present no evidence against this assumption.

In Figure 2 the age distribution is given of scrapie cases in females observed in private flocks. The relatively small Suffolk sample from our records is shown together with published information on a larger sample which includes various breeds (Parry, 1962). Both samples are fairly similar in showing the highest frequency of observed cases in the three-year old sheep. And both samples include cases in fairly old sheep.

Provided that the average incidence of scrapie, irrespective of age, is low (less than 10 percent) and that the above conditions regarding culling apply, then the observed incidence of scrapie in each age of sheep can be corrected to allow for the reduced proportion of the original group still at risk. In this way a more valid estimate of the age distribution of scrapie can be obtained. This corrected distribution for the pooled data is shown in figure 2.

Two useful conclusions can be drawn from this. Although scrapie most commonly appears when the ewe is a young adult there is no ground for regarding an old sheep as free from the disease or resistant to it, but it develops less frequently in older ewes. Furthermore, so far as the usefulness of field data are concerned, this interpretation of the evidence demonstrates the difficulties involved: less than half the sheep survive their fourth year but only three scrapie cases out of four have been observed to develop the disease by that age. Table 1 shows the difference in this respect between the original data and the culling-corrected data. The error rate involved in classifying animals on their scrapie-status up to 4 1/2 years is only one in ten for the uncorrected data whereas the real error rate is in the order of one in four in these samples.

[1] Animal Breeding Research Organization, Edinburgh, Scotland.
[2] Director, Animal Diseases Research Association, Moredun Institute, Edinburgh, Scotland.
[3] Clinical Investigation Department, Animal Diseases Research Association, Moredun Institute, Edinburgh, Scotland.

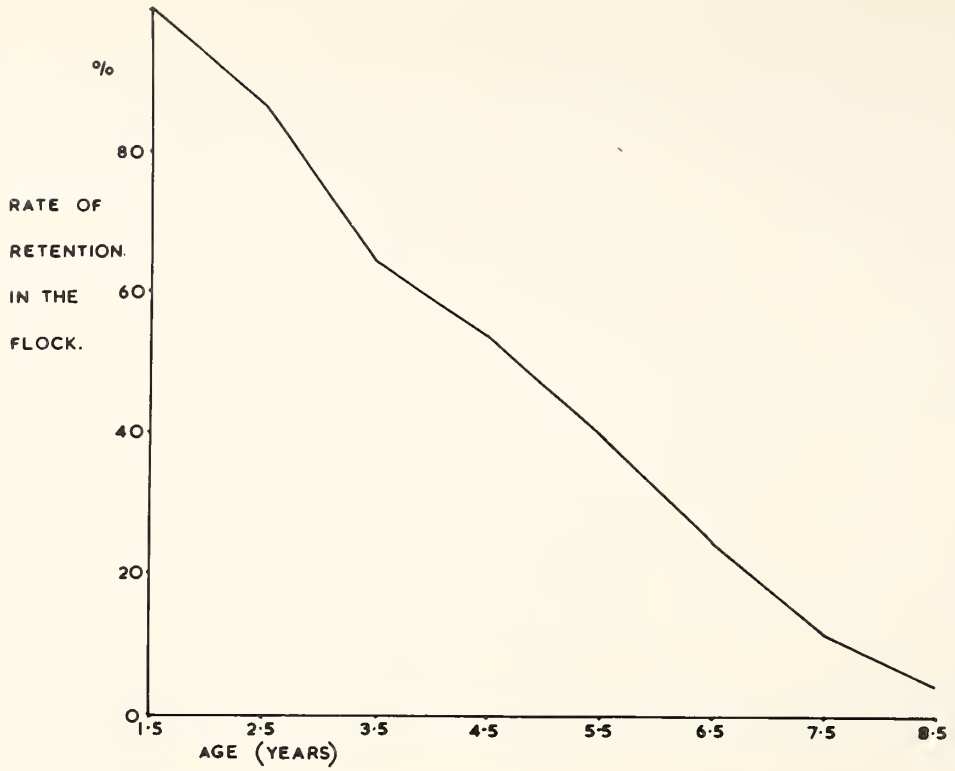


Figure 1.--The effect of culling and death on the age composition of a sample of Suffolk flocks.

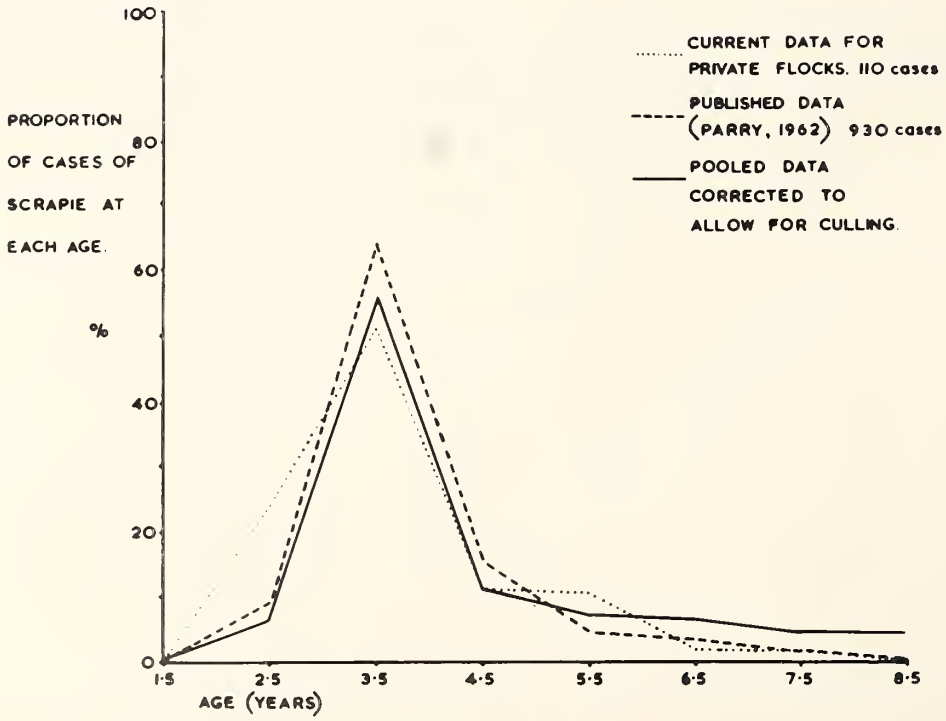


Figure 2.--The age distribution of scrapie cases in private flocks.

Table 1.--Proportion of scrapie cases which have developed by $4\frac{1}{2}$ years of age

Item	Current data	Parry (1962)	Overall data
	Percent	Percent	Percent
Original data.....	85	89	89
Data corrected for culling.....	71	76	76

It is interesting to note that a similar estimate of 74 percent based on 28 animals has recently been used by Draper and Parry (1962): this figure contrasts markedly with the 90 percent estimate previously applied to an extensive series by Parry (1962).

The majority of data on scrapie in the general sheep population are for females, for obvious reasons. Hence the discussion above has referred only to the ewes but there is no evidence to suggest any fundamental differences between the sexes in their liability to scrapie and it seems reasonable to expect the deductions given here to be applicable to rams as well as ewes.

The question of why there is a peak incidence of the disease in young adults can only briefly be touched on and it must be speculative in the present state of knowledge. Much depends on whether or not scrapie is caused by an independent pathogen. The balance of evidence, some to be brought out in subsequent papers, favours the presence of a pathogen. If this is so the age distribution will depend on the following factors:

(1) The incubation period considered as a characteristic of the scrapie agent.
(2) The age at which the sheep encounters the pathogen.
(3) The possibility that resistance to the pathogen may change with the age of the sheep either for developmental or immunological reasons.
(4) The possibility that some sheep may modify the incubation period. This possibility will be the subject of a later paper and will not be amplified till then.

The incubation period is known to be prolonged and can be variable according to circumstances over a wide range extending at least from 4 months to 42 months. It is certainly puzzling why scrapie in sheep hardly ever occurs before 2 years old. However, the inferred age distribution is consistent with the hypothesis that most victims encounter the pathogen either prenatally or in early post natal life resulting in the peak incidence at 2 or 3 years old, and that resistance to the agent may increase after this early period of development, the low incidence of cases in older sheep being at least partly accounted for by successful pathogen attacks encountered in adult life. On this view genetical variation, seen most simply as susceptibility or resistance, may act in determining the rate at which resistance increases with age from conception onwards. The rationale for some of these postulates will emerge when details of our experimental sheep matings are given.

REFERENCE

Draper, G. J. and Parry, H. B. (1962) Nature 195: 670.
Parry, H. B. (1962) Heredity 17: 75.

DISCUSSION

Mr. Draper:

I want to comment on this difference between the two manifestation levels. I did make this point yesterday. I said that one of them was conditional on observing the disease; I said one was conditional on the animal being alive at the age of 9 months. Dr. Dickinson heard this. It was explained what the difference was and why we made this difference which was because from Parry's you cannot make predictions. Whether or not the theory is right or wrong, this point was made very clearly, that we changed the definition of this manifestation level because we wanted to do something different with this. This is quite irrelevant to the question of whether the theory is right or wrong.

I want to make the point that we said yesterday what was put on the board just now. Not only that we said it 18 months ago and we said in the paper we published that this definition differs from one used previously.

Dr. Dickinson:

You did not explain why you had changed in your paper, in Nature, nor did you explain how this biased, very seriously, all of the previous evidence which Parry alone had presented in his interpretation of the disease. It seemed as though you were changing for some transient reason or one that was more appropriate to the experimental work that you were involved in then, and this had no necessary and destructive effect on the previous data on which this recessive gene had been founded.

Mr. Draper:

If you seriously wanted to know the reason why we made this difference in definition, it was there 18 months ago in cold print; you could have written to Nature or written to me.

Just as you have been collecting data which seems to point in a certain direction, I suggest this is what Parry did before. We wanted to do something more precise and this is why we did this.

I have defined here how we got a difference of manifestation level. You have told us, in what you put on the board, that you have corrected the observed figures. Perhaps we could know how you made these corrections? This is a technical point admittedly. It doesn't affect our arguments.

Dr. Dickinson:

Simply by using a linear 10 percent per annum decrease rate of survival within the flocks. This is the crudest approximation at which we can arrive. It seems to be broadly true for our own data and for the Suffolk breed as a whole and for the Leicester breed. Can you suggest any alternative?

Mr. Draper:

This is not really an explanation; it merely shows how you made this correction. One would like to see it written out in more detail.

Dr. Dickinson:

Just take one age group where 50 percent only have survived to that age, and you observe a 10 percent incidence in that group. That is 10 percent. Certainly if you double this, you still ought to arrive at a 10 percent incidence. But you now have twice as many; you will then have twice as many actual observed cases. I am not talking about percentages now. So if this had been 40 cases here, you arrive back at 80 cases and you accumulate numbers, not percentages. It is simply by accumulating numbers in that form and arriving back and taking this as an overall total and working out for each age your percentage of this total corrected number.

Could we just have a clear statement from Mr. Draper whether he considers my method and motive in correcting the cumulative age incidence data as appropriate and it should be done or not. If it is appropriate and should be done, they should have done it; if it is inappropriate, we must discuss why.

Mr. Draper:

I do think this is appropriate, that is why I did it 18 months ago.

Dr. Dickinson:

You did this for--I think there were 37 cases that had then gone to 3 years old, or 2 years perhaps, and 17 cases, or perhaps 19 cases, that have gone to 3 years old.

Now I am not talking about these little groups. I want us to put this back into Parry's lap in terms of data which he says prima facie support a recessive gene hypothesis. It knocks that to pieces, not your 1962 paper on a very few sheep, which I have no criticism of or comment about whatsoever.

Mr. Parry:

I must thank you, Mr. Chairman, and ask you to thank your Department for at least allowing us to understand Dr. Dickinson. He probably did say this at the Veterinary Research Club meeting, but in the way it was said, or something, we failed to understand fully what he was getting at.

We now do see how he is arriving at this criticism. I would like the meeting to know that Dr. Dickinson, I think, should have received a manuscript of my Heredity paper from Professor Mather at least 6 months before it was published.

Dr. Dickinson:

This I did.

Mr. Parry:

I didn't know, because I had no acknowledgement of this receipt. No comment has reached us. We are very interested to know this, because after all, if I may be perfectly frank, the Heredity paper was an attempt to clarify the basic background. If there are defects in the reasoning and so on, I personally want to know about it so we can go back.

The detailed data that Mr. Draper presented yesterday, supporting or otherwise, the genetic hypothesis, particularly the table relating to the paper in Nature of 1962, was obtained in a flock with a very different situation from the breed average. In this flock, first of all, no females are being culled for any reason other than physical unsuitability for breeding, at my request, in the years under review, and no ewes are culled whatever. They die of various competing risks. And I might add that they only live to 9 or 10 years, when they come to me for autopsy, and it is not physically possible to take them beyond 10 years.

The figures for the Suffolk breed, knowing the Suffolk breed perhaps rather well, I should hesitate to make any general inferences from data obtained from the British Suffolk flock book, because it is only in the last 5 or 6 years that female pedigrees have been recorded, partly I think as a result of my activities in the breed.

These data have variable reliability and it is far from complete. There are 1400 registered Suffolk flocks in the British Suffolk Book. We are in fact only concerned with 30 to 40. And the figures quoted for the whole breed, I doubt whether they are representative. I can't tell you at the moment, but in most of my flocks, for example, we would have, as I showed you yesterday, in many of the flocks, nearly 15 percent of ewes age seven or over.

Dr. Dickinson:

Is this now or was this in the period from say 1950 to 1957?

Mr. Parry:

You have no figures for 1950-57.

Dr. Dickinson:

Yes, we do have. I am not using the Flock Book.

Mr. Parry:

There are no records in the Suffolk Flock Book.

Dr. Dickinson:

I am not using the Flock Book. I am saying this happens to measure up to one broadly based sample for a broadly similar breed. If these figures each deviated by 10 percent either way, it wouldn't make much difference. But the situation in the last 5 years is quite different within the Suffolks. I have used the Suffolk Flock Book for one figure only. My colleague, Doctor Wiener, did this some years back. It shows 5,000 to 6,000 new females enter the Suffolk breed each year. That is the only thing I said about the Suffolk Flock Book this morning for data use.

Mr. Parry:

Yes. The point is the linear age loss rate over five, it may be true as a speculation, what we want to do is establish it for the flocks with which we are dealing. We have this information.

Dr. Dickinson:

And you have not used it. This is my criticism.

Mr. Parry:

We have in fact got this data in the Flock Book such as the one on exhibition yesterday, which you are all welcome to look at for a great number of flocks. We haven't set about correlating it, because this is quite a prodigious task.

Dr. Dickinson:

It is not.

Mr. Parry:

It can be done and now we know the substance of Dr. Dickinson's objection, I am sure Mr. Draper will see we do what he considers necessary statistically.

Dr. Dickinson:

You must also be aware of the particular question of the genetically circular argument. Any geneticist would say this is the hole that all amateurs fall into genetically and you have fallen in. And this is the substance of my criticism.

Mr. Parry:

I don't think this age factor being discussed affects the analysis of the critical crucial information, which was on the table given by Mr. Draper yesterday afternoon, namely, about the incidence of scrapie in sire-progeny groups. Nor does it affect the data in my matings, nor does it affect the observations in one flock in which I have an affected ram mated with ewes, which I think are clear; and none of the progeny have gone down. It is, of course, possible they might have gone down at 25 years of age.

Mr. Yohe:

When you are correcting your factor, something about culling unrelated to scrapie, this I am a bit curious about, as to what happens in a culling program of a breeder of Suffolk or any sheep in Britain. It would seem to me, knowing what has been said here, that if such an outbreak occurs, he is very apt to cull not only the individual, but the progeny and the sire and dam. What is the general practice in Britain?

Dr. Dickinson:

I think this is a little difficult to answer, because I think Mr. Parry can show how different situations occurred in some of his flocks. I think this will come out. One breeder who, when he started culling against scrapie, flock A again, his proportion of young animals has gone up. This is his policy quite markedly. Yesterday I think Mr. Parry had one flock where he either said this or we saw data on this, you had a slide where the opposite had occurred, culling for scrapie in that particular situation had resulted in an increasing preponderance of the older age groups. Now I can see how both these policies can be quite rational if one wants to do these things. One can either say I will have a bigger turn-over rate in my flock and try to cull out as soon as one case occurs early enough in that family or quite the opposite experience and this is the one that I think possibly an odd person or two has been successful with, but I think they are running risks, of saying right, I will keep my ewes until as old as possible and breed all my replacements from very old animals. This, I think, must be the substance of the situation here. In this case, you are going against trying to get rid of scrapie by high turnover rate. But I think it is a possible policy. But as we have seen, flock A was culling out relatives already in the flock of animals that were affected. I think he is on the less safe of these two alternatives.

Mr. Yohe:

By relatives, you mean offspring?

Dr. Dickinson:

And keeping proportionately more younger animals coming in. He has a fairly fertile flock, so he is in fact able to do some culling of females normally. But the difficult point here is that while applying this policy, it rids him of the possibilities of culling for other things to a very large extent. I think this other policy, that is, the practice of keeping one animal and observing it for many years; and when it doesn't go down with scrapie, therefore you say you will get replacements from it, but this decreases your ability to select for growth or something else you are interested in just because of the lower turnover rate. But I think it is odd how these two policies are in fact being used and implemented because of the relative prolificacy of Suffolk sheep under some circumstances. The breeder must choose how many collateral relatives, how many distant relatives to cull out and what risks he is willing to take on new progeny coming in from new rams which he doesn't know anything about. This is the risk that the people keeping the older stock try to avoid.

Mr. Yohe:

Apparently flock A was doing this on more or less a flexible basis.

Dr. Dickinson:

I think he was forced to be flexible, because as I kept stressing, he had these cases widely dispersed in this flock--if he whole-hogged it and got rid of more distant relatives, instituted the kind of policy which presumably is leading to an older age grouping, bang go his 200 ewes. This is his dilmena. If you in fact think you see within your flock scrapie being largely in this kind of breeding line and not in another, you cull around one group and the others must be allowed to grow older. But this breeder was having to cull out all over the place. So it probably meant he couldn't get rid of cousins or aunts and uncles. And this in fact gave a younger age

structure to his flock because of having to keep a higher proportion of ewe lambs to maintain his flock size.

Mr. Parry, is this unusual in your experience, that culling for scrapie produces a higher proportion of younger animals?

Mr. Parry:

Well, it can be. If you have an old ram that goes down when he has many daughters in the flock, age 3 and up, and you decide you want to cull, then you introduce new young animals and this does distort the situation. On the other hand, if one is lucky, the scrapie appears in the ram before many of his daughters are in the flock, then culling his progeny causes much less disturbance of age composition.

Dr. Dickinson:

This was difficult, as I said the 21 rams Flock A used, none of them are gone with scrapie. A lot may depend on whether rams develop scrapie for each particular flock owner or not.

Mr. Parry:

Yes, at what age the rams go. This is terribly critical. You get all sorts of patterns.

Paper No. 26

THE PATHOLOGY OF SCRAPIE

I. Zlotnik [1]
(Presented by J. T. Stamp) [2]

INTRODUCTION

The present paper summarises the observations made over several years regarding the histology and pathology of a very large number of animals; 500 normal sheep, 50 normal goats, 500 normal mice, 6 normal hamsters, 900 sheep of various breeds and 5 goats affected with natural scrapie, and 730 sheep, 259 goats, 8,000 mice and 18 hamsters affected with experimentally produced scrapie. Many of the findings have already been published (Zlotnik, 1957, 1958a and b, 1960, 1961, 1962a and b, 1963; Zlotnik and Rennie, 1957, 1958, 1962, 1963; Zlotnik and Stamp, 1961, 1962) but additional information is now included.

It has been shown that the only pathological changes that can be demonstrated in scrapied animals are in the central nervous system and the only consistent and constant lesions are in the brain. The spinal cord may be affected but to a lesser extent and the lesions, when present, are often very unevenly distributed and are thought therefore to be of little diagnostic value (Wight, 1960).

BRAIN LESIONS

The only naked eye change in scrapie animals consists of an increase in the cerebrospinal fluid and this is found in about 80 percent of affected animals. The amount of fluid found in the cranial cavity of sheep affected with scrapie varies from 100 to 200 ml.; it is clear and shows no chemical or cytological abnormality.

The essential lesion of scrapie is microscopic, non-inflammatory in character and consists of a focal or diffuse degeneration of the grey matter of various parts of the brain.

[1] Pathology Department, Animal Disease Research Association, Moredun Institute Edinburgh, Scotland.
[2] Director, Animal Disease Research Association, Moredun Institute, Edinburgh, Scotland.

Neuronal changes are the most striking and involve either a few or almost all of the neurones of any particular brain nucleus. Severe change is also present in the perineuronal grey matter in the form of status spongiosus and this is especially obvious in sheep and goats following prolonged clinical symptoms but in laboratory animals status spongiosus is seen even in the early stages of the disease. Animals killed in advanced stages show hypertrophy of astrocytes and occasionally myelin degeneration especially of the medulla and cord.

The changes in the neurones vary from simple chromatolysis to pyknosis, sclerosis, necrosis and vacuolation. Vacuoles in the neurones occur constantly and are of various shapes and forms; they are highly characteristic of scrapie. They may appear as clearly demarcated empty spaces in an apparently healthy cytoplasm or may form single or multiple cavities in cells undergoing other forms of degeneration. Within the vacuoles round, eosinophilic bodies may be present or the vacuoles may be filled partly or completely with granular necrotic debris (figures 1 to 6 and 9).

Status spongiosus is a common occurrence in scrapie brains. It is common in areas of widespread neuronal destruction but is also seen in the mesencephalon, diencephalon and telencephalon of sheep and goats without any accompanying neuronal degeneration. In scrapie-affected mice and hamsters status spongiosus is often widespread not only in the subcortical centres of the brain but also in the cerebral cortex. The extent of the lesion varies from only slight perineuronal oedema to almost complete destruction of the brain substance. The microscopical appearance of these advanced lesions resembles a network of fibres and strands with only a very few neurons remaining (figures 7, 8, 10, 11 and 12).

Proliferation of astrocytes is thought to occur in advanced cases of scrapie but since the number of astrocytes in normal brains have not been counted it is uncertain whether the apparent increase in scrapie is real or represents hypertrophy and oedema of existing cells. There is no doubt that the astrocytes seen in areas of severe status spongiosus are very hypertrophied and it is possible that fluid accumulates in the astrocytes thus causing distention and increase in size of both cell bodies and protoplasmic processes.

Although the lesions of every scrapied animal have a similar appearance, differences do occur in the distribution and intensity of the pathological changes.

DISTRIBUTION OF BRAIN LESIONS

Natural Scrapie of Sheep

Irrespective of breed, lesions are always present in the subcortical centres of the brain but not all parts are either always or equally affected. The cerebral cortex is never affected. In general, the severity of clinical symptoms is reflected in the intensity and distribution of brain lesions but cases have been seen where this was not so.

Various breeds of sheep differ in the extent of lesions found, for Cheviots appear to have less severe lesions than do Swaledales and Border Leicesters while Suffolks are the most severely affected (figure 13). The medulla is invariably affected in all scrapie sheep but while the pons and mesencephalon of scrapie-affected Suffolk sheep always have lesions, in only 75 percent of Cheviots and 80 percent of Swaledales are these areas affected. Differences are also found in the tegmental nuclei of the mesencephalon-pons junction, cerebellum, thalamus, hypothalamus and paraterminal body where lesions are present in from 10 to 85 percent of cases.

While lesions can be found in many parts of the brain the most commonly affected centres are situated along the midline of the neuraxis. Thus lesions are always present in the formatio reticularis alba and the reticular formation of the medulla while in the pons the tegmental nuclei and the reticular formation are predilection sites. In the mesencephalon the tegmental nuclei and the pigmented midline cells are often affected and in the anterior parts of the midbrain the paraventricular nucleus of the hypothalamus and the paraterminal body of the telencephalon are the centres most likely to show lesions. In addition to the nuclei along the midline of the neuraxis, other nuclei show lesions. In the medulla the cuneate nuclei (cuneate and lateral

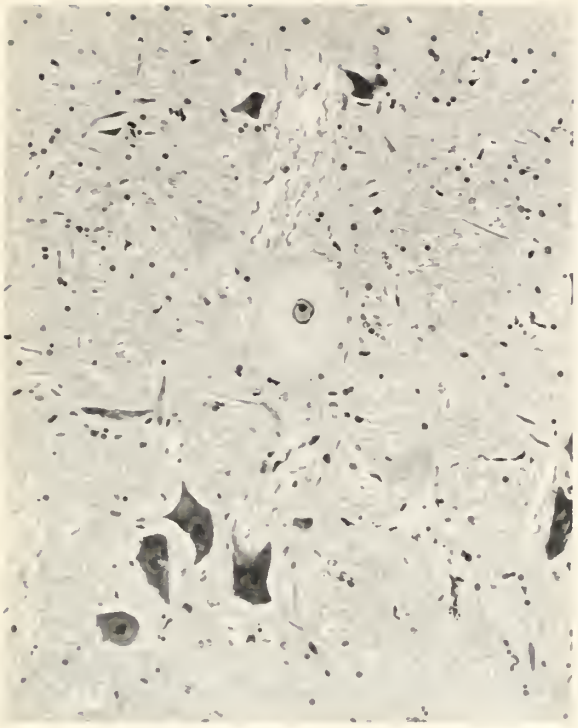


Figure 1.--Chromatolysis and pyknosis in the red nucleus of Suffolk sheep--natural scrapie. (X 200.)

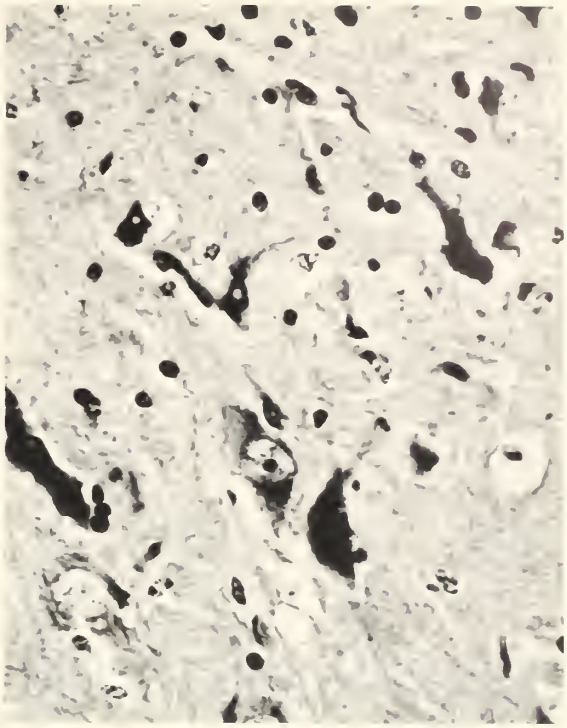


Figure 2.--Pyknosis of ganglion cells in the hippocampus of goats--drowsy syndrome of scrapie. (X 440.)

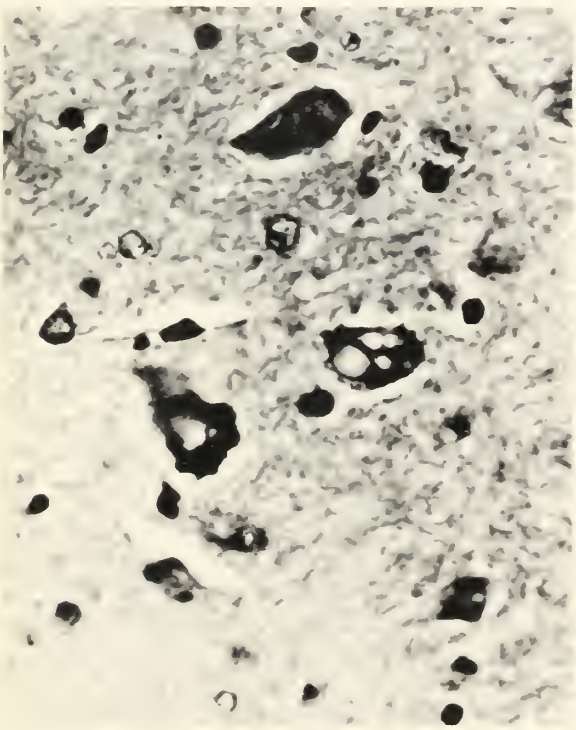


Figure 3.--Sclerotic neurones containing multiple vacuoles in the paraterminal body of goats--uninoculated contact goats. (X 680.)

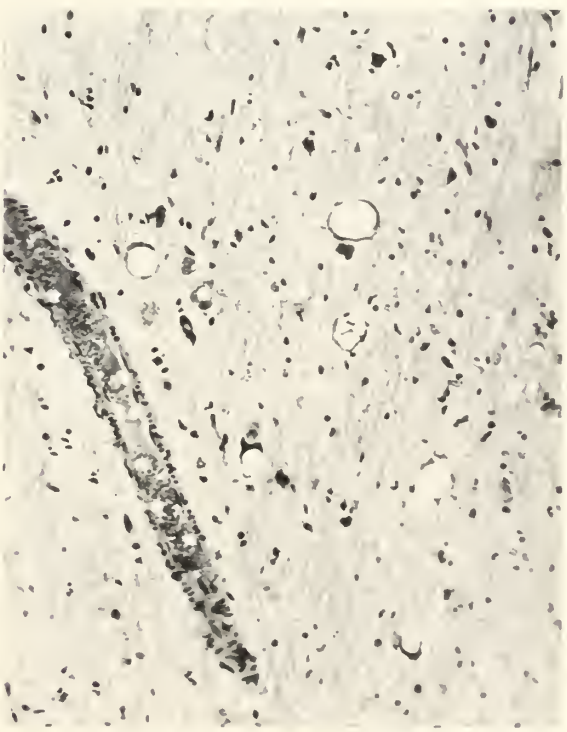


Figure 4.--Vacuolated neurones in the inferior colliculus of goats--scratching syndrome of scrapie. (X 180.)

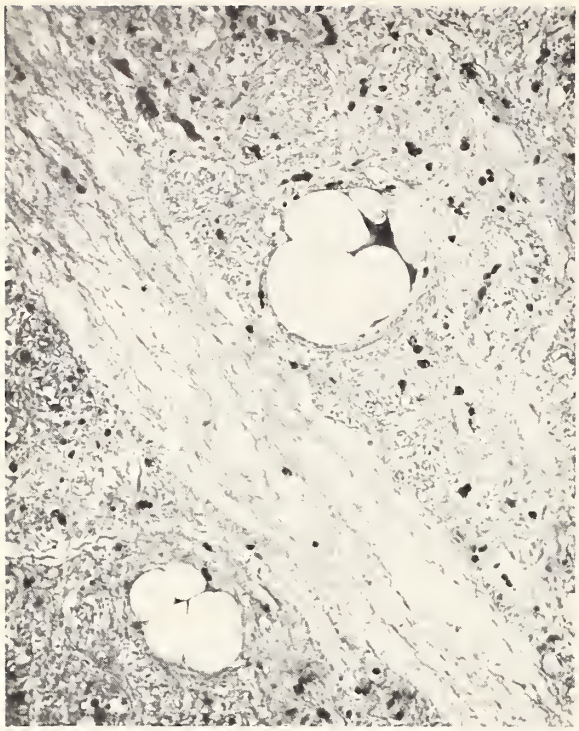


Figure 5.--Giant vacuoles in the red nucleus of goats--scratching syndrome of scrapie. (X 180.)

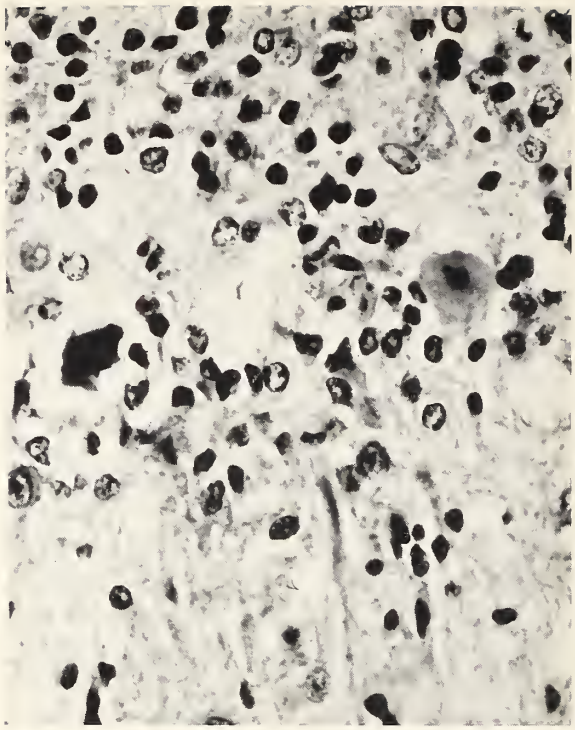


Figure 6.--Degeneration in the granular layer and in Purkinje cells of the cerebellum of Suffolk sheep--natural scrapie. (X 520.)

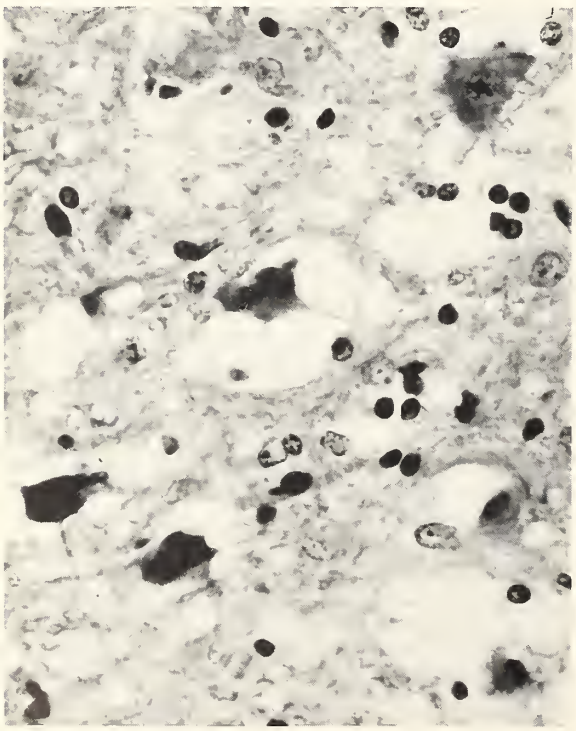


Figure 7.--Status spongiosus in the thalamus of goats affected with the drowsy syndrome of scrapie. (X 520.)

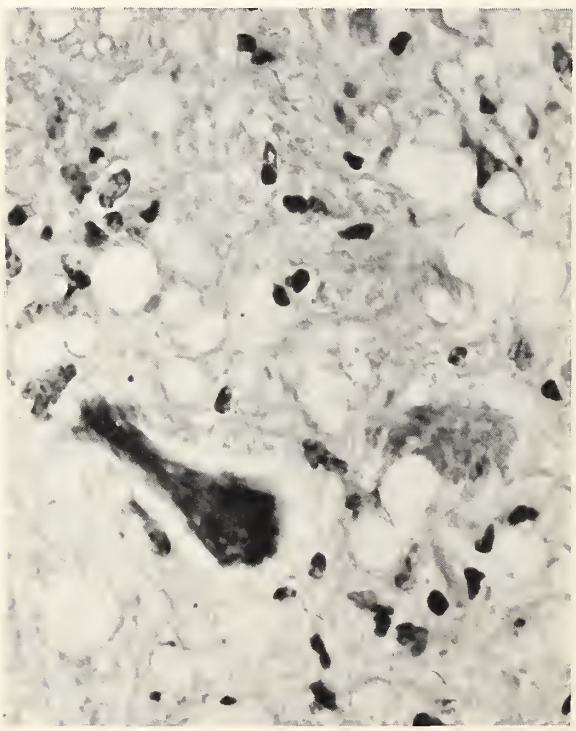


Figure 8.--Status spongiosus in the mesencephalon of Suffolk sheep--experimental scrapie. (X 600.)

Figure 9.--Vacuolation of the lateral vestibular nucleus in hamsters affected with scrapie. (X 110.)

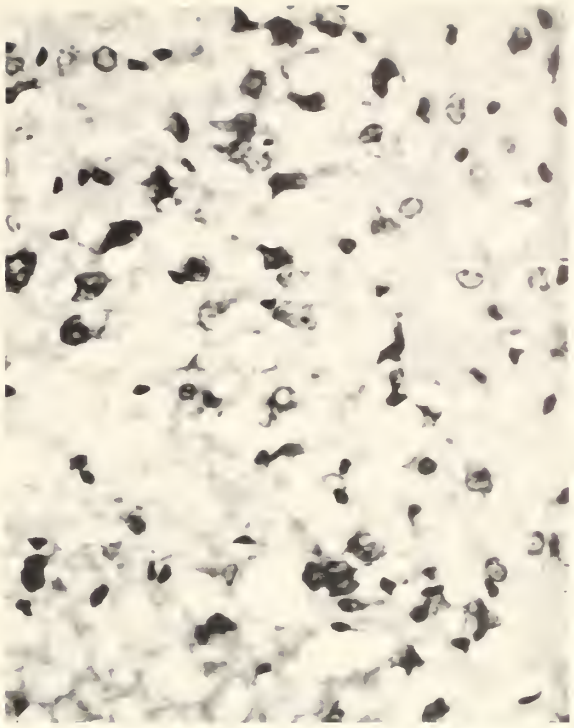


Figure 10.--Status spongiosus of the mesencephalon in mice affected with scrapie (scratching goat strain). (X 440.)

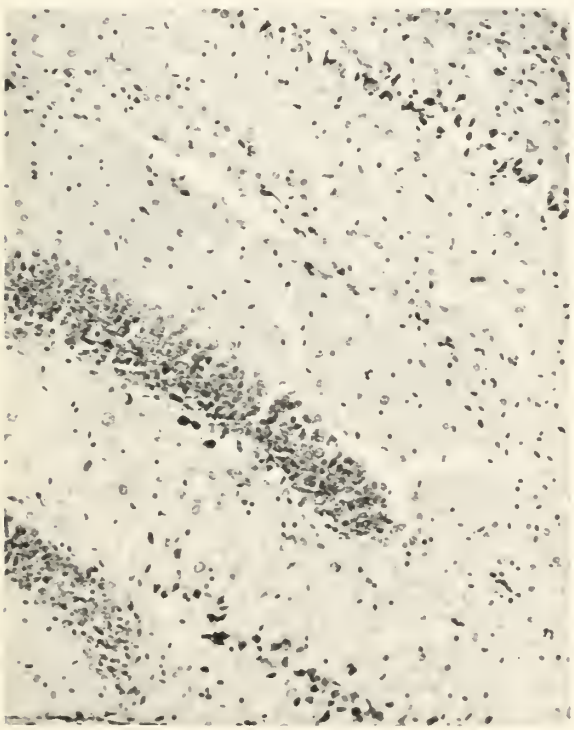


Figure 11.--Status spongiosus in the hippocampus of mice affected with scrapie (Suffolk sheep strain). (X 150.)

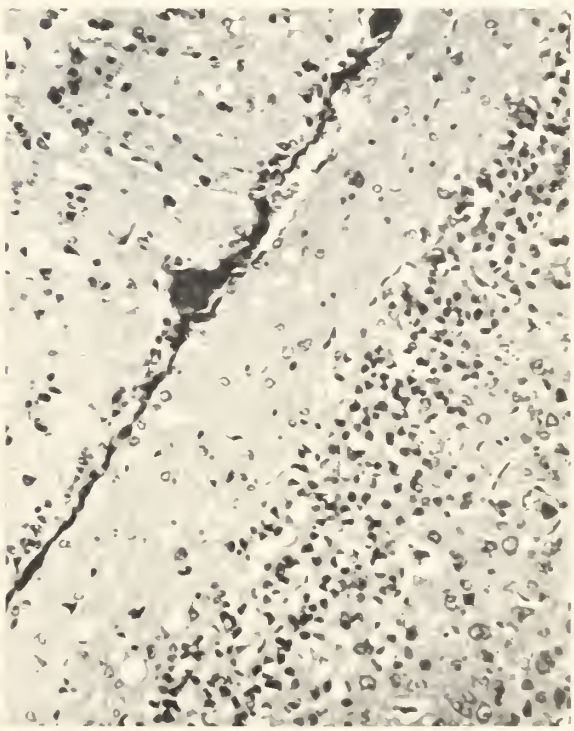


Figure 12.--Status spongiosus of the cerebral cortex of hamsters affected with scrapie. (X 180.)

CEREBRAL LESIONS IN SHEEP AFFECTED WITH SCRAPIE.

		Medulla		Pons		Tegmental nuclei Mes—Pons.		Mesencephalon		Thalamus.		Hypothalamus.		Paraterminal Body.		Cerebellum.	
		Inc. %	Ext.	Inc. %	Ext.	Inc. %	Ext.	Inc. %	Ext.	Inc. %	Ext.	Inc. %	Ext.	Inc. %	Ext.	Inc. %	Ext.
Natural Scrapie.	Cheviots.	100	+ +	75	+	25	+	75	+	20	+	50	+	10	+	25	+
	Swaledales.	100	+ +	80	+ +	30	+	80	+ +	30	+	60	+ +	25	+	25	+
	Suffolks.	100	+ + +	100	+ + +	60	+	100	+ +	50	+	85	+ +	50	+	70	+
Experimental Scrapie.	Cheviot sheep inoc. with Cheviot scrapie brain. I.C. route very early clin. cases	100	+	25	+	Nil	—	Nil	—	Nil	—	Nil	—	Nil	—	Nil	—
	Cheviot sheep inoc. with Cheviot scrapie brain. I.C. route clin. cases	100	+	100	+	25	+	75	+	25	+	60	+	Nil	—	25	+
	Cheviot sheep inoc. with Cheviot scrapie brain. Sub-cut. route clin. cases	100	+ +	100	+ +	Nil	—	50	+	10	+	10	+	Nil	—	10	+
	Cheviot sheep inoc. s/c with Suff. scr. brain. Clin. cases	100	+ +	100	+	100	+ +	100	+	100	+	50	+	100	+ +	30	+
	Suff. sheep inoc. I.C. & s/c with Suff. scr. brain. Clin. cases	100	+ + + +	100	+ + +	100	+ +	100	+ +	75	+ +	100	+ + + +	75	+	70	+ +

Inc.— Incidence (% of brains containing lesions) Ext.— Extent of lesions + limited, often confined to one brain nucleus.
++ moderate, present in more than one nucleus
+++ severe.
++++ very severe and widespread.

Figure 13.--Cerebral lesions in sheep affected with scrapie.

cuneate) are invariably and severely affected in all well-established clinical cases of scrapie in Suffolk sheep but are less often so in Swaledales and only very rarely in Cheviot sheep. In the pons the vestibular nuclei, and in the mescencephalon the red nucleus are frequently affected. The corpora quadrigemina also contain lesions but the superior colliculus is less often affected than the inferior. Lesions are present also in the substantia nigra, geniculate bodies and thalamic nuclei, the incidence varying from 20 percent in Cheviot to 50 percent in Suffolk sheep. In the cerebellum disappearance of the granule cells and thinning of the granular layer are seen while the neurones of the cerebellar nuclei and the Purkinje cells also undergo degeneration. Because of the great number of cerebellar folia, lesions of the cerebellum as a whole are not always readily found. In about 25 percent of affected Cheviots and 70 percent of Suffolks both degeneration of Purkinje cells and granular layer cells as well as proliferation of Bergmann nuclei are found (figure 6). In the hypothalamus the supraoptic nucleus and nuclei of the mammillary body show pathological changes while in the paraterminal body both the gyrus subcallosus and the area parolfactoria contain lesions.

It is important to point out that the types of lesion as well as their distribution vary between the different breeds. In the Cheviot neuronal lesions predominate and only slight astrocytic changes occur since status spongiosus is very rarely seen. Even if present the extent of the status spongiosus is very slight and only found in the diencephalon and telencephalon. In the Suffolk, on the other hand, status spongiosus is always present in advanced clinical cases and can be seen in the medulla and pons while in the mesencephalon and thalamus it may be very extensive.

Experimental Scrapie in Sheep

In general, the distribution of brain lesions in experimentally affected sheep is similar to that of sheep of the same breed suffering from the natural disease but in Cheviot sheep the intensity and severity of lesions is less while in the few Suffolks experimentally inoculated with Suffolk scrapie brain, and subsequently developing symptoms, lesions are very severe and widespread (fig. 14).

Cheviot sheep inoculated intracerebrally with Cheviot scrapie brain and destroyed soon after the appearance of first symptoms all have lesions in the medulla while 25 percent also have changes in the pons while other parts of the brain are not affected. Vacuolation of neurones, chromatolysis, and pyknosis are the only changes in the nuclei of the midline of the medulla, in the reticular formation and in the formatio reticularis alba. In the pons the neurones of the lateral vestibular nuclei and of the nuclei of the midline are degenerate. On the other hand, when similar sheep are allowed to develop advanced symptoms the lesions become more widespread so that lesions also occur in the tegmental nuclei, mesencephalon, hypothalamus, thalamus and cerebellum. A comparison of the lesions which follow intracerebral inoculation with those that follow subcutaneous inoculation shows that although the total number of vacuolated neurones in the medulla, pons and mesencephalon is similar their distribution differs considerably. In the intracerebrally inoculated animals the most common sites of lesions are the nuclei of the midline of the medulla and the tegmental matter of the pons-mesencephalon and the nuclei of the diencephalon and telencephalon while in those subcutaneously inoculated the arcuate nuclei and the inferior olive are the centres more commonly affected.

The incidence and distribution of lesions in Cheviot sheep inoculated with scrapie brain of the same breed differ from those of Cheviot sheep given Suffolk scrapie brain and which develop scrapie 6 to 9 months later. Brain lesions in these latter animals are not, as a whole, very severe but although none of the animals was allowed to develop very advances symptoms neuronal lesions are widely found being always present in the medulla, pons, tegmental nuclei, mesencephalon, thalamus and paraterminal body and, on occasion, in the hypothalamus and the cerebellum. In addition to neuronal degeneration status spongiosus is always present being especially obvious in the paraterminal body and less so in the thalamus and mesencephalon. The cuneate nuclei did not show neuronal vacuolation but slight status spongiosus was evident around the neurones which were as a rule pyknotic. The inferior olive and the arcuate nuclei had lesions similar to those of Cheviot sheep inoculated subcutaneously with Cheviot scrapie brain.

The brains of Suffolk sheep that developed scrapie 15 months after intracerebral and subcutaneous inoculations of Suffolk scrapie brain show very severe and widespread neuronal degeneration and vacuolation, hypertrophy of astrocytes and very extensive status spongiosus. The medulla, pons, mesencephalon, hypothalamus and tegmental nuclei of the mesencephalon-pons junction are all affected as also are the cerebellum, paraterminal body and thalamus in many cases. Of special interest is the presence of very severe neuronal lesions along with status spongiosus in the cuneate nuclei of the medulla, centres so far found unaffected in Cheviot sheep inoculated with brain from scrapied Cheviots.

Experimental Scrapie in Goats (figure 14)

(a) Goats inoculated by the subcutaneous route with the scratching type of scrapie.--Goats destroyed before symptoms appear (7 to 12 months after inoculation) always have lesions in the medulla but only about half show changes in the mesencephalon and in only a quarter is the pons affected. In the medulla slight status spongiosus is also present in the inferior olive and pyknotic cells are scattered in the raphé and recticular formation. Vacuolated neurones are present in the arcuate nuclei.

Animals destroyed soon after the appearance of clinical symptoms (9 to 14 months following inoculation) have more widespread and severe lesions. The medulla and pons are regularly affected, 70 percent of such goats have lesions in the mesencephalon and 50 percent in the tegmental nuclei of the mesencephalon pons. The hypothalamus and paraterminal body are only very occasionally affected. The arcuate cells of the medulla are vacuolated or chromatolytic.

In inoculated goats allowed to develop advanced symptoms severe lesions are always found in the medulla and pons. Nearly all the arcuate cells are affected in the medulla as also are the neurones of the formatio reticularis alba, reticular formation, inferior olive and dorsal motor vagus. Significant changes are

CEREBRAL LESIONS IN GOATS AFFECTED WITH SCRAPIE

		Medulla.		Pons.		Tegmental nucl. Mes-Pons.		Mesencephalon.		Thalamus.		Hypothalamus.		Paraterminal Body.		Cerebellum.	
		Inc. %	Ext.	Inc. %	Ext.	Inc. %	Ext.	Inc. %	Ext.	Inc. %	Ext.	Inc. %	Ext.	Inc. %	Ext.	Inc. %	Ext.
Goats inoc. i/c with scratching strain of scrapie	Pre-clinical cases.	100	+	25	+	10	+	50	+	Nil	—	Nil	—	Nil	—	Nil	—
	Very early clinical cases.	100	+ +	100	+ +	50	+	70	+	Nil	—	30	+	10	+	Nil	—
	Clinical cases.	100	+ + +	100	+ + +	70	+	70	+ +	30	+	70	+ +	50	+	10	+
Goats inoc. i/c with scratching strain of scrapie	Pre-clinical cases	80	+	30	+	100	+	30	+	Nil	—	Nil	—	50	+	Nil	—
	Very early clinical cases	80	+	100	+	100	+ +	100	+ +	20	+	20	+	100	+	Nil	—
	Clinical cases	100	+	100	+ +	100	+ + +	100	+ + +	80	+	100	+ +	100	+ + +	20	+
Goats inoc. i/c with drawsy strain of scr Clinical cases.		100	+	100	+	100	+ +	100	+ + + +	100	+ + + +	100	+ + + +	100	+ + +	70	+
Uninoc. goats in prolonged contact with scrapie Suff. sheep. Clinical cases.		100	+ + + +	100	+ + + +	100	+ + + +	100	+ + +	100	+ +	100	+ + +	100	+	75	+ +

Figure 14.--Cerebral lesions in goats affected with scrapie.

not seen in the cuneate nuclei. In the pons lesions are present in the raphé, reticular formation, vestibular nuclei, spinal nuclei and pontine nuclei. The tegmental nuclei of the mesencephalon-pons are affected in 70 percent of animals. In the mesencephalon the red nucleus, the pigmented cells and the inferior colliculi have changes in 70 percent of cases and the nuclei of the hypothalamus are similarly affected. Other areas of the brain are affected to a lesser degree.

(b) Goats inoculated by the intracerebral route with the scratching type of scrapie.-- In preclinical cases destroyed 6 months or more after scrapie inoculation lesions are always found in the tegmental nuclei of the mesencephalon-pons junction. The lesions are not very severe and are found mainly in the dorsal tegmental nucleus and to a lesser extent in the nucleus centralis superior and the nucleus motor-tegmento-peduncular. Lesions in the medulla, mainly in the raphé, are present in 80 percent of animals and 50 percent of such goats have changes in the paraterminal body. The pons and mesencephalon rarely show lesions and no abnormality is present in the cerebellum, hypothalamus and thalamus. In goats killed showing early symptoms there is an increased severity and incidence of lesions in the same areas. In goats allowed to develop advanced symptoms all parts of the subcortical brain show lesions with the exception of the thalamus where changes are present in 80 percent of cases and of the cerebellum where only 20 percent of brains have lesions. Status spongiosus occurs regularly but is moderate in extent and confined mainly to the mesencephalon, diencephalon and telencephalon. In the medulla neuronal lesions are not marked and are limited to the nuclei of the midline, the cuneate nuclei being unaffected. The most severe lesions, both neuronal and extraneuronal, are present in the tegmentum, the inferior colliculi, the red nucleus and the gyrus subcallosus.

(c) Goats inoculated by the intracerebral route with the drowsy type of scrapie.-- Lesions are seen in all the subcortical centres of the brain with the exception of the cerebellum where lesions are present in only 70 percent of goats. The lesions are much more severe than those of any of the preceding groups of goats and status spongiosus is very widespread throughout the subcortical brain with the exception of the cerebellum where it is seldom found and of the medulla and pons where it is limited in extent. The most severe changes are found in the mesencephalon, diencephalon and telencephalon. In the mesencephalon the large neurones of the red nucleus contain very big multiple vacuoles or they are completely necrotic. The cells of the

substantia nigra and the inferior and superior colliculi are often affected while the lateral geniculate body and the thalamic nuclei are severely affected with status spongiosus. The mammillary body, the paraventricular nucleus of the hypothalamus and the supraoptic nucleus also show very severe degenerative changes.

The least affected parts of the brain are the medulla, pons and cerebellum while the tegmental nuclei of the mesencephalon-pons are somewhat less severely affected than the same centres of the brains of goats affected with the scratching type.

Natural Scrapie in Goats

Five cases of scrapie in uninoculated goats have been seen. One of these is a control animal which was kept for 6 to 7 months in close contact with goats inoculated with scratching goat scrapie and which developed scrapie before symptoms were observed in the inoculated animal (Mackay and Smith, 1961). The other four goats had been housed for about four years since they were a day old with numerous scrapie-affected sheep mainly of the Suffolk breed (Stamp, 1962).

In the brain of the control goat (kept with other inoculated goats) lesions are present which resemble, both in distribution and in severity, those seen in goats inoculated subcutaneously with the scratching type of scrapie and killed soon after clinical symptoms developed. Lesions are present in the medulla, pons, mesencephalon and hypothalamus. There is little tendency towards spongy degeneration, the neurones being mainly affected. Changes are not seen in the cuneate nuclei of the medulla but the nuclei of the reticular formation and the arcuate cells are affected. In the hypothalamus the mammillary body shows the most extensive lesions.

The four goats housed in very prolonged contact with scrapied sheep mainly of the Suffolk breed have brain lesions resembling those seen in Suffolk sheep. The majority of neurones of the medulla and medulla-cord junction are vacuolated and degenerated, the vacuoles being mainly in necrotic or chromatolytic cells or of the multiple variety. In the cuneate nuclei as well as degeneration of neurones there is also a big loss of neurones along with widespread status spongiosus which extends into the reticular formation. In the pons severe degeneration of neurones occurs in many nuclei including the pontine nuclei where most of the neurones are vacuolated. Severe degeneration of neurones and status spongiosus is present in the mesencephalon, diencephalon and telencephalon. The most severe changes in the cerebellum are present in the granular layers where widespread loss of cells is very evident.

Scrapie in Mice

Following the successful transmission of scrapie to mice from goats affected with the drowsy syndrome of scrapie (Chandler, 1961, 1962, 1963 and Pattison and Smith, 1963) the disease has been transmitted to mice from goats affected with the scratching syndrome, from scrapied Suffolk sheep and from Southdowns affected with scrapie (Zlotnik and Rennie, 1962, 1963). Lesions in mice are confined to the brain and, as with scrapie in sheep and goats, neurones, extraneuronal substance and astrocytes are affected. The brain pathology of mice differs, depending upon the origin of the scrapie inoculum whether it is first-passage material from sheep and goats or not, and upon the type of scrapie inoculum.

In mice inoculated with the drowsy type of goat scrapie on first passage pathological changes in the form of oedema of the white matter and moderate status spongiosus of the grey matter can be observed in the medulla, pons, hippocampus, mesencephalon and hypothalamus. The astrocytes show hypertrophy especially in areas affected by spongy degeneration. Definite neuronal vacuolation is not found in the subcortical centres situated along the neuraxis. On second and further passage an intensification of these pathological processes occurs. Status spongiosus affects most parts of the brain including the hippocampus, the cerebral cortex, the corpus striatum and the thalamus. In the cortex, in areas of very severe status spongiosus, many neurones are vacuolated or pyknotic.

Mice inoculated with either the Suffolk or Southdown sheep strain or with the scratching

goat type of scrapie have all on first passage similar lesions. Vacuolation and degeneration of the neurones of the brain stem are always present in contrast to the drowsy type, and, in addition, there is status spongiosus of the anterior parts of the neuraxis. Vacuolated neurones are regularly found in the raphé of the medulla, in the lateral vestibular nucleus of the pons, in the tegmental nuclei of the mesencephalon and in some of the Purkinje cells of the cerebellum. Extra-neuronal vacuolation is present not only in areas where neurones are degenerating but also in the hypothalamus, thalamus, hippocampus and paraterminal body. On second and early passages an intensification and extension of the pathological process occurs similar to that seen in mice inoculated with the drowsy strain of scrapie but neuronal vacuolation remains very conspicuous especially in the lateral vestibular nuclei. In the later passages the lesions tend to concentrate in the more anterior portions of the brain including the cerebral cortex. Severe damage, including neuronal vacuolation and status spongiosus is seen in the cortex, thalamus, hippocampus, hypothalamus, corpus striatum, paraterminal body and olfactory lobes (figures 10 and 11) but the medulla and pons, and in some cases the mesencephalon, are less severely affected. In some brains of these later passages the medulla and mesencephalon are normal and in the pons only the vestibular nuclei have vacuolated neurones.

Scrapie in Hamsters

Scrapie in hamsters has been produced by the intracerebral inoculation of mouse-passaged Suffolk sheep strain or by mouse-passaged scratching goat strain. The lesions in the hamster brain on first passage consist of very prominent vacuoles in the neurones of the lateral vestibular nuclei along with neuronal degeneration and status spongiosus in all other parts of the brain including the hippocampus and the cerebral cortex. It appears therefore that the first passage of scrapie to hamsters produces both cerebral and subcortical lesions whereas in mice lesions are confined to the subcortical centres.

CONCLUSIONS

The paper draws attention to the fact that the pattern and the distribution of scrapie lesions in the brain of sheep vary according to the breed of the animal and/or upon the type of inoculum used to produce the disease. The pathology of scrapie in the sheep would suggest that there are in sheep at least two distinct types of scrapie, the Suffolk and the Cheviot. This differentiation of scrapie on morphological grounds is supported by the evidence of experimental transmission to mice, for the transmission of scrapie from Suffolk sheep to mice is comparatively easy whereas the transmission from Cheviot sheep, either naturally or experimentally affected, is difficult.

The lesions in Cheviot sheep, whether naturally or experimentally affected, are confined mainly to the neurones of the brain stem with little or only moderate change in the anterior parts of the brain. On the other hand, the brain lesions in scrapied Suffolk sheep are usually very severe and widespread and consist of neuronal degeneration, astrocytic change and status spongiosus. The telencephalon, diencephalon, pons and medulla are markedly affected, as also are the cuneate nuclei, these latter centres seldom being affected in the brains of scrapie-affected Cheviots.

Two types of scrapie differentiated on clinical grounds have been described in goats (Pattison and Millson, 1961). As described in the present paper, lesions appear to be more severe in goats affected with the drowsy strain than in goats affected with the scratching strain. The two strains of scrapie in the goat however can be clearly differentiated by sub-inoculations of both types into mice where each produces a somewhat different clinical syndrome following a different incubation period and with distinct pathological features.

The naturally affected scrapie goats described in the paper fall into two groups. The first group consists of a single uninoculated goat which developed scrapie after being housed for 6 months with a group of goats inoculated with the scratching type of goat scrapie. The lesions in the brain of this goat are similar to those described in goats as a

result of the subcutaneous inoculation of the scratching strain while subinoculations of this brain into mice produced a disease syndrome similar to that seen when mice are inoculated with the scratching goat strain. The second group consists of 4 uninoculated goats kept in very prolonged contact with numerous scrapied sheep mainly of the Suffolk breed and which developed scrapie after 3 1/2 to 4 years. The brain lesions of all four animals are similar in every respect to those present in scrapied Suffolk sheep and this suggests that the disease in the goats is an example of true contact infection from Suffolk sheep to goats.

Although, as a whole, the morphological changes seen in all experimentally affected animals are similar to those seen in the natural disease, subcutaneous inoculation appears to reproduce the pathological pattern of the natural disease more closely than does intracerebral inoculation.

REFERENCES

1. Chandler, R. L. (1961. Lancet, i, 1378.
2. Chandler, R. L. (1962). Ibid, i, 107.
3. Chandler, R. L. (1963). Res. Vet. Sci., 4, 276.
4. Mackay, J. M. K. and Smith, W. (1961). Vet. Rec., 73, 344.
5. Pattison, I. H. and Millson, G. C. (1961). J. Comp. Path., 69, 360.
6. Pattison, I. H. and Smith, K. (1963). Res. Vet. Sci., 4, 269.
7. Stamp, J. T. (1962). Vet. Rec., 74, 357.
8. Wight, P. A. L. (1960). J. Comp. Path., 70, 70.
9. Zlotnik, I. (1957). Nature, 180, 393.
10. Idem (1958a). J. Comp. Path., 68, 148.
11. Idem (1958b). ibid, 68, 428.
12. Idem (1960). Nature, 185, 785.
13. Idem (1961). J. Comp. Path., 71, 440.
14. Idem (1962a). ibid, 72, 366.
15. Idem (1962b). Acta Neuropath. supp., 1, 61.
16. Idem (1963). Lancet, ii, 1072.
17. Zlotnik, I. and Rennie, J. C. (1957). J. Comp. Path., 67, 30.
18. Idem (1958). ibid, 68, 411.
19. Idem (1962). ibid, 72, 360.
20. Idem (1963). ibid, 73, 150.
21. Zlotnik, I. and Stamp, J. T. (1961). World Neurology, 2, 895.
22. Idem (1962). Proc. IV Int. Cong. Neuropath., 3, 468.

DISCUSSION

Question:
What part does the site of inoculation play in the distribution of lesions?

Dr. Stamp:
I am sorry, I cannot answer the question. All I can say is that the inoculation is made, more or less, into the center of the frontal bones.

Dr. Dickinson:
All my mouse inoculations are done laterally into the brain, yet the lesion is bilaterally symmetrical.

Dr. Plummer:
Dr. Stamp, do you recognize that sheep die of scrapie, die suddenly, without having previously exhibited signs and if you recognize such, what is the pathology in the animal?

Dr. Stamp:
We have seen scrapied sheep die suddenly this way. I wouldn't say after not exhibiting any signs, but not exhibiting signs which we thought were of scrapie, especially in Suffolk sheep. And in the Suffolk sheep of this type that we have examined, the pathology is that which I have described. The lesions are

generally very florid. In other words, extreme, very massive and very marked lesions are present.

Have you had the same sort of experience, Dr. Plummer?

Dr. Plummer:

Yes. What is disturbing is if this is true, you would think the extent of the lesions didn't have very much effect on the animal, because as you say the animal appears to be quite normal.

Dr. Stamp:

Yes. I would agree entirely. In my presentation I was very careful to qualify the statement that on the whole lesions corresponded to clinical signs, but I said not always. And especially does this go for Cheviot sheep.

Dr. Mikel:

Am I correct in understanding that there are four strains of scrapie agent?

Dr. Stamp:

Yes. Dr. Gordon will help me here possibly. We recognize a Cheviot strain and we recognize what we call a Suffolk strain. These are two distinct sheep strains. We also recognize a drowsy strain in the goat and a scratching strain in the goat. The drowsy strain and the scratching strain in the goat originate from Welsh mountain sheep inoculated with Cheviot material. How these two strains came about is a little confusing. The two strains came for some unknown reason, and Mr. Pattison of Compton was a sufficiently skilled clinician to realize it.

Dr. Burger:

Dr. Stamp, I must say your presentation and your slides have removed from my mind the last doubt that the mink disease we have observed in Northern Wisconsin, not only clinically but also pathologically resembles scrapie rather closely.

Dr. Stamp:

I have seen some of the photographs from Wisconsin. I think it will be of considerable interest to the English visitors when we hear your paper and see the photographs. The lesions in the mink are in fact very spectacular.

Mr. Parry:

How many sections did you do? How did you sample, or how did Zlotnik arrive at this sample? He can't have looked at every section.

Dr. Stamp:

He has a definite routine in which he divides the brain up into numerous segments, I think in the region of 40 or 50 segments, and he takes a definite number of sections from each segment of each part of the brain.

Mr. Parry:

In view of what I said yesterday, has he noticed any particular location in particular nuclei as distinct from these general areas?

Dr. Stamp:

This information is in the paper, Mr. Parry. I am sorry. I didn't really wish to weary everyone with the detail. I was a little worried someone was going to ask me where the various brain nuclei were and--I have a picture of them if you would like to see it.

Dr. Mulhern:

Dr. Stamp, from some of the discussion yesterday on vacuolization, I felt it was implied by you that there is no other disease in sheep in which this pathology has been observed.

Dr. Stamp:

That very definitely is so. I would say that during a very extensive experience of sheep pathology, I have never seen anything in sheep that resembles the pathology of scrapie except scrapie. There are a number of other experienced neuropathologists here, perhaps they would like to comment on this.

A "SCRAPIE"-LIKE DISEASE OF MINK

D. Burger[1] and G. R. Hartsough [2]

Evidence gathered in cooperation with Dr. G. R. Hartsough Great Lakes Ranch Service, Pittsville, Wis., indicated the presence of an apparently new disease among ranch-raised mink.

It represents a chronic disorder of the central nervous system characterized by progressive debility and locomotoric incoordination. The course of the disease extends over several weeks and ends almost invariably in death. At present there is no evidence for spontaneous spread among mink.

During July 1963 the malady was brought to the attention of our laboratory by Dr. Hartsough. Necropsies performed on dead or euthanized affected animals were characterized by a conspicuous absence of lesions, except for an occasional slight reddening of the meningi. Bacteriologic examination of the major organs did not yield any specific pathogens such as listeria. Only a few colonies of E. coli and nonhemolytic staphylococci developed along the inoculation streaks.

Clinical, pathologic, and histologic examination did not produce evidence for neurotropic distemper.

Aleutian disease of mink was excluded on grounds of absence of typical pathologic changes. Moreover, iodine agglutination tests for hypergammaglobulinemia performed with sera of 3 affected mink were negative.

Introcerebral or intraperitoneal inoculation of filtered or unfiltered brain suspensions into suckling and weanling white mice and guinea pigs did not reveal the presence of a pathogen. However, the experiment was terminated after an observation period of 2 weeks.

Peritoneal washings and impression smears from brain material of infected mice were stained with giemsa and examined for toxoplasma.

All slides were negative.

Embryonated eggs and tissue cultures prepared from mink kidneys, mink testicles, calf kidneys, calf testicles, chick embryos and HeLa cells failed to show cytopathic effect during an observation period of 6 - 14 days.

We were fortunate to get Dr. Hadlow interested in this unusual disease. He visited the Hayward ranch, collected specimen on his own and in his laboratory has studied the histopathology of this disease.

Therefore, only a few of our epizootiological data shall be presented here.

In June 1963 the stricken mink herd consisted of 1,270 breeder mink and about 4,500 mink kits.

During the first half of 1963 the health record of the Hayward ranch was exceptionally good. According to Dr. Hartsough's files some losses had occurred in January due to a food-borne anaerobic infection, apparently due to Cl. welchii. The losses stopped soon after treatment with antibiotics began.

Vaccinations against Cl. botulinum, virus distemper, and mink enteritis had been omitted by the rancher, thus excluding the possibility of a syringe borne disease.

The losses were confined entirely to breeder animals. The first signs of the disease were seen by the owner as early as June with deaths occurring in the same month.

During July and August the losses averaged about 12 to 15 animals a day. On October 8 only 12 of the 1,270 adult mink were still alive many of them already showing signs of incoordination and feebleness. During the months June through October a total loss of 99 percent of the adult animals accured, but none of the 4,500 kits, although held in very close contact with the breeder mink, contracted the disease.

The high morbidity made feed suspect as possible source of the disease. Since none of

[1] Fellow, United States Public Health Service (Training Grant 5 TIAI 1750 4); Department of Veterinary Science, University of Wisconsin, Madison, Wisconsin.

[2] Veterinarian in Charge, Diagnostic Laboratory, Great Lakes Mink Association, Pittsville, Wisconsin.

the kits were affected, we assumed, that the faulty feed was consumed by the adult mink alone, i.e. before the young mink were born.

An inspection of the premises and feed processing facilities on the ranch eliminated the possibility of heavy metal poisoning.

Since a good portion of the feed fed on the Hayward ranch was made up of commercial products, many of them fed on other ranches, our search concentrated on unusual ingredients present in the ration fed on the Hayward ranch.

The records showed feeding of carcasses of locally trapped beavers during the months of March and April. No unusual beaver mortality occurred during the winter 1962/3 in the opinion of our wildlife specialists.

Another lead was more promising, however: When investigating the fate of 9 male mink sold by the Hayward ranch to the Fall Creek ranch, 90 miles south of Hayward, it was learned, that all 9 mink had died or were stricken by a similar disease as present on the Hayward ranch. In addition, an undetermined number of adult mink had also died on the Fall Creek ranch.

Moreover, during September 1962 a truck load of mink feed, which, according to the seller consisted of beef carcasses unfit for human consumption (so-called downer cows), was shipped from the Fall Creek ranch to the Hayward ranch. Meat from the same sources was also fed on the Fall Creek ranch.

Since mink on both ranches showed identical signs of disease in our opinion, this shipment, reportedly consisting of carcasses of downer cows, has to be incriminated.

The possibility of introducing the disease on the Fall Creek ranch by the shipment of 9 mink mentioned before is, in our opinion, at variance with the observation of healthy kits present on both ranches.

In the absence of any other clues, we are inclined to assume the presence of an as yet hypothetical, contagion in the incriminated shipment of carcasses. Considering the largely degenerative changes in the brains of affected animals, the apparently long incubation period (6 months or more) this hypothetical contagion could bear some resemblance to Scrapie of sheep.

However, in the absence of experimental evidence for a viral etiology of this disease or its experimental transmissibility to sheep or goat, this hypothesis remains rather speculative.

About 4 months ago we have started a series of transmission experiments using filtered and unfiltered mink brains. One of these 20 mink met accidental death rather early. All other mink are in good health.

Experimental transmission to mice has not been successful as yet. As soon as the weather conditions will permit inoculation of experimental sheep and goats will be initiated under strict quarantine.

In closing I would like to mention, that a similar disease was observed in fall 1963 by Dr. Hadlow on a mink ranch in Idaho. According to Dr. Hartsough's files a similar disease was present on isolated Wisconsin mink ranches in the past. One case deserves particular emphasis, since it demonstrates again the possibility of an unusual long incubation period. In fall 1948, 125 mink were shipped from a mink ranch in Wisconsin to another ranch in Minnesota. The disease occurred simultaneously on the ranch of origin and in the animals shipped. A spread to other mink was not observed.

DISCUSSION

(A Motion Picture of Affected Mink Was Shown.)

Dr. Gordon:

Did you observe this animal scratching itself with its hind legs?

Dr. Burger:

We are not certain. If you test the animal and try to get a nibbling response or reflex, whatever you want to call it, the only thing is, it bites you. It may very well be. I am not certain. You will have to make up your mind from your experience with sheep. The only thing we can say, we never saw that in mink before.

Dr. Gordon:

It has a very strong resemblance to an encephalopathy, such as one sees in the small animals to which scrapie has been transmitted. Perhaps it is not inappropriate to say that if

subsequently it is proven to be scrapie, this might produce some alleviation on the ban on the importation of British sheep to a country which has already got scrapie. You haven't so far inoculated sheep, goats or mice?

Dr. Burger:
Only mice.

Dr. Gordon:
How long have the mice been inoculated?

Dr. Burger:
I discontinued the experiment for the following reasons: We have had a outbreak of an unknown disease in the mouse colony, after which I felt it would be appropriate to discontinue and start over again, because I wouldn't be sure what I was transmitting.

Dr. Stuart:
You said there was degeneration. Was vacuolation found?

Dr. Burger:
Not definitely--in the neurons.

Dr. Hadlow:
Dr. Burger mentioned that I had visited a ranch in Idaho where this disease had also occurred. This outbreak occurred just about a year ago. In this case the losses from death were less, but the great loss was sustained by not getting a kit crop, since the loss occurred before the breeding season. There is a similarity in the microscopic changes in the brain, with those of scrapie. But I wouldn't call it scrapie. The distribution of the lesion is somewhat different from what we were told this morning about the distribution of the lesions of scrapie, but it is essentially the same type of degenerative change in which this spongy state occurs. The spongy state is one of the distinguishing features as well as a marked astrocytosis. As for vacuolation of neurons, one can see these occasionally, but one is not impressed with their abundance.

The lesions seem to be most severe in the rostral parts of the brain, characteristically in the cerebral cortex. That of the frontal lobe is most severely affected.

It also occurs in the corpus striatum. As one moves caudally in the brain, the lesions are less severe. I haven't seen any lesions in the spinal cord.

There is this similarity as far as the lesion is concerned in that here, too, we are dealing with not an encephalitis but with a degeneration of the brain.

Mr. Parry:
Am I right that the kits were fed an entirely different diet?

Dr. Burger:
No. Two weeks after they are born they are taking feed. So the diet consumed by kits and adults I would figure from the end of May was the same, although the quantities consumed may have been different for adults and kits.

Mr. Parry:
Any Lake Michigan fish in the diet?

Dr. Burger:
I wouldn't presume they had been fed Lake Michigan fish.

Dr. Stamp:
How long were the kits kept before being killed? I think it is important to know the difference in time period between the age of the kits and the age of the mature mink.

Dr. Burger:
I do not know the exact date of pelting on that ranch. I would assume it was shortly after Thanksgiving. However, since all the old breeders were lost, the new breeders were recruited from the kit crop. So we may have a chance to observe a thousand animals for at least another year, and we may even be able to observe offspring of these kits which were entirely bred in a closed colony again. How long we can continue that, I don't know.

Dr. Goode:
I want to make one brief announcement before we move on. Mr. Linn has some printed material that he wanted to get into the hands of each member of the group that will be serving on the evaluation panel tomorrow and the following day. It amounts to two different items here at the end of this table. We would appreciate it if you would stop by and pick those up.

SCRAPIE IN SUFFOLK SHEEP: A GENETICAL INVESTIGATION USING EXPERIMENTAL MATINGS

A. G. Dickinson[1], G. B. Young[1], J. T. Stamp[2], and C. C. Renwick[3]
(Presented by A. G. Dickinson)

From what is already known of the agent which causes scrapie it is clear that certain types of breeding experiment cannot be carried out satisfactorily. There are a number of reasons for this. The natural disease is of widespread distribution and, because of its insidious nature and variable clinical expression, laboratory confirmation of diagnosis is needed, thus making it difficult to assemble a group of sheep reliably free from the disease. In addition, the scrapie agent has a high degree of chemical and physical resistance to sterilising procedures which could be applied to premises and equipment. In consequence it is impracticable to undertake experiments which can only be interpreted on the assumption that the environment or host are absolutely free from scrapie. Therefore, until recently, any direct experimental attack has been impossible on the important question of whether contact transmission from sheep to sheep occurs under natural conditions: this particular work is still in an early stage and no results can be given.

Eight years ago when the experimental breeding to be described was started, the dominant purpose was to verify the belief commonly held by breeders that scrapie outbreaks often follow a familial pattern. Experiments were designed to cover among others, the possibility that a simple genetic mechanism, such as a recessive or dominant gene, caused scrapie without the involvement of a pathogen. The analysis of this work has lead to the conclusion that neither a dominant nor a recessive gene can be the sole cause of the disease and that the involvement of a pathogen responsible for scrapie must be assumed. The experiments have shown that the breeders observations of a familial pattern of appearance of scrapie were well founded and a likely biological explanation for this has been established. The aim of this paper is to present the data which point to these conclusions.

CRITERIA FOR SCRAPIE USED IN THE EXPERIMENTS

For reasons which will be obvious from other papers, the classification of experimental breeding stock as affected or unaffected cannot be judged from observations alone and the need to keep all animals until they are about to die has also been dealt with. All animals dying over one year old in these experiments have been checked by neuropathological examinations and any which died in circumstances preventing brain examination have had to be discounted or are specifically indicated as such. Indeed, this reliance on neuropathology has brought to light a number of early cases of the disease which were not detected on clinical grounds - about 10 percent of the scrapie cases originated this way.

SOURCES OF BREEDING STOCK

The experiments can be considered in two phases, the assembly of foundation stock and the production of progeny from experimental matings. The approach has been to acquire

[1] Animal Breeding Research Organization, Edinburgh, Scotland.
[2] Director, Animal Diseases Research Association, Moredun Institute, Edinburgh, Scotland.
[3] Clinical Investigation Department, Animal Disease Research Association, Moredun Institute, Edinburgh, Scotland.

two groups of Suffolk sheep: animals directly related to a case of scrapie form one group, the other comprising animals unrelated for at least a few generations to scrapie cases and from flocks where the disease had not been known to occur for many years. The two groups are referred to respectively as OS - "off-scrapie" - and OF - "off-free".

The easiest and most reliable group to assemble were cases with an affected dam. Many of these came in as lambs being nursed by their affected mother - hence the problem of inaccurate flock pedigree records was virtually bypassed. This latter problem must be greatest with progeny from affected sires but this particular type only constitute 40 percent of the OS group and for reasons which will emerge they have contributed little to the subsequent analyses. Only 6 percent of the OS stock are known to have both parents affected. Nineteen flocks of origin are represented in the OS group, with a combined annual total of about 600 adult sheep during the years of close observation. With limited records and the complications of culling it is difficult to arrive at an accurate and meaningful estimate of the incidence of scrapie. As an approximation, in these 19 flocks, ewes had about a 10 percent chance of developing scrapie during their lifetime in the flocks.

The search for OF stock raised certain difficulties. To have included stock from flocks with uncertain histories of supposed freedom from scrapie would be pointless. Our conditions for inclusion had to be stringent and involved an assessment of the reliability of flock records, the trustworthiness of the owner and his ability to have recognised scrapie had it occurred; four flocks meeting these conditions have been involved. Stock from a fifth flock which was vouched for by an independent person and included because of our shortage of OF stock were initially present among the OF group: a full enquiry subsequent to the appearance of a case of scrapie brought sufficient evidence to light to necessitate the culling of this entire group. The four flocks have totalled about 600 adults during the years they have been under observation and scrapie has not appeared in any of them. However, despite the careful screening, scrapie could have occurred unnoticed in these flocks, for reasons already given. Therefore as an initial proving period, OF ewes were kept loosely isolated from OS ewes for 3 1/2 years after purchase and during this time no scrapie was seen to develop among stock from the four flocks. The isolation simply amounted to grazing in separate fields, which for the OF stock had no recent history of being used for sheep; unavoidable contact came from the flock group which was later excluded, mating with OS rams and indirectly via the same stockman, sheepdogs and vehicles attending both groups.

INCIDENCE OF SCRAPIE IN FOUNDATION STOCK

Figure 1 shows the age incidence data for the three types OF, OS (dam affected) and OS (sire affected). Animals where both parents are known to be affected are excluded.

The OS (sire affected) group has an average incidence of 25 percent among animals at least 4 1/2 years old or having developed scrapie before that age. This average value cannot form the basis of any deductions since one of the sires has a much higher proportion of affected

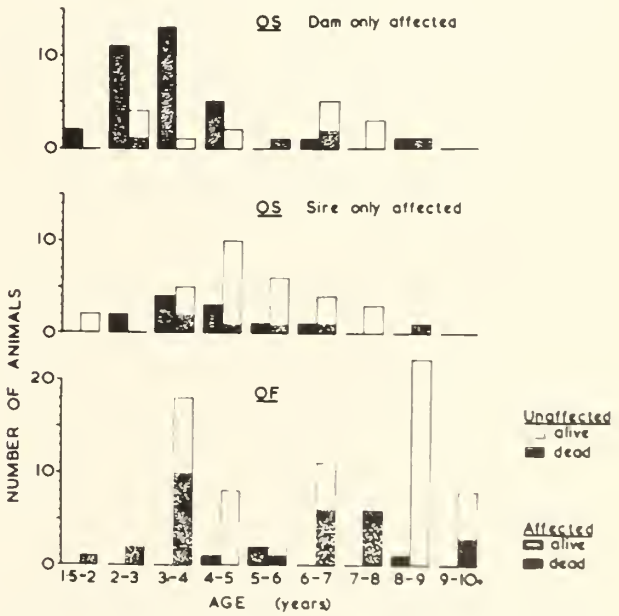


Figure 1.--The distribution of affected & unaffected Suffolk sheep mainly ewes, in purchased foundation stock

offspring than the others (70 percent and 12 percent respectively: $P < 0.02$). It is tempting to try to explain away this apparent heterogeneity in terms of unreliable pedigree records associated with some of the sires so giving us the wrong lambs but no adequate evidence has been found for this. Alternatively, it may be contended that for the deviant sire group several of the dams were actual or potential scrapie cases but unrecognised as such. There is no particular reason why this should be so but it is not impossible. However, there is some evidence of similar results from experimental matings, which adds to the cogency of the above as being a valid case of sire heterogeneity but no firm conclusions can be drawn.

With the OS (dam affected) group the incidence among stock born at least 4 1/2 years ago has reached the high level of 67 percent. The implications of this are dealt with after the OF group has been discussed.

Among the OF group, no case of scrapie has occurred before 4 1/2 years old. Of the four cases which developed later there are good grounds for excluding two. One was the only egg donor we have and was operated on contemporaneously with scrapie stock: the surgical procedures could easily have produced an experimental challenge with scrapie agent. The other was one of four OF animals placed at 2 years old in maximum lifetime contact with cases of natural scrapie. Excluding these two special cases, the OF incidence is 5 percent. It is especially noticeable that the age distribution of scrapie cases in the OF group is quite different from that in the OS group. Although there could be several explanations for this, the difference is of the type which would be expected if contagion were involved and the OF group had not encountered the pathogen until a much later age than the one at which OS stock encountered it. The low incidence and its confinement to older sheep is regarded as support for the assessment of the flocks of origin as being virtually free from scrapie.

SCRAPIE INCIDENCE IN PROGENY OF OF EWES

The unaffected OF ewes have been mated with one or more of ten Suffolk rams which have varying relationships with affected animals. A summary of the outcome of these matings is given in figure 2, the purpose of which is to show the proportion of OF ewes which have left at least one affected offspring. The more progeny a ewe has, the more reliable will be the estimate of the proportion which can leave affected offspring. From figure 2 it is concluded that at least half of them can do so, since 49 percent of this sample left an affected lamb even including ewes with only a single observed offspring. If a recessive gene were the sole cause of the disease there would be no alternative but to deduce that at least half of the OF stock were heterozygotes, most of the remainder being dominant homozygotes and a few, say 5 percent, being homozygous recessives which were not seen to develop the disease. Such a mixture is hard to reconcile with population theory unless an assumption of homozygote inviability is resorted to and for which there is no independent evidence whatsoever. Failing this the high frequency of presumed heterozygotes in our OF sample should be matched by a scrapie incidence on the farms of origin in the order of 25 percent and this is not so.

The results shown in figure 2 do not rule out other simple genetic explanations such as a dominant gene but for reasons which will emerge the most likely explanation is in terms of a pathogen, not encountered on the 4 farms but present for some or all of the experimental progeny.

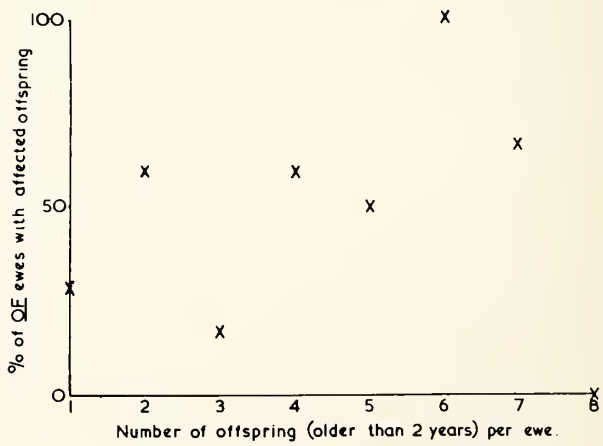


Figure 2.--The presence of scrapie in the progeny of 37 unaffected OF ewes, classified according to progeny group size

INCIDENCE OF SCRAPIE IN OS ANIMALS

A similar conclusion stems from the OS (dam affected) data but for quite different reasons. It is impossible to reconcile the high incidence of scrapie with the notion of a recessive gene causing scrapie unless certain qualifications are introduced, since 50 percent is the highest Mendelian expectation possible, even after making the unlikely assumption that all unaffected rams were heterozygotes - and the observed 67 percent deviates even from this ($P < .05$). If this hypothesis is to be retained for further consideration it must be assumed that a proportion of the male parents on the farms of origin, all thought to be unaffected, have been misclassified and were in fact potential or actual cases of scrapie and were homozygous recessives. It is quite feasible that this has happened in some cases but, in the light of the generally low incidence of scrapie and the specific exclusion of rams known to be affected, it is unlikely that misclassification occurred frequently. Also, since the ram progeny group size in the OS (dam affected) class is rarely more than two, occasional misclassification of the rams can only have a very limited effect. When these data, with allowance for misclassification are considered in terms of known laws of population structure for a recessive gene, the outcome is shown in figure 3. The term "misclassification rate" requires some explanation: it denotes the proportion of postulated homozygous recessive rams in any particular population which have been wrongly classified as unaffected: the absolute number of animals misclassified for a given sample size varies with the allele frequency.

It can be seen from figure 3 that as the extent of misclassification rises the recessive allele frequency falls, thereby reducing the average incidence of scrapie expected in the general population on the farms of origin, in terms of this model. At the most unlikely extreme of 100 percent misclassification, the average incidence is expected to be almost 50 percent of the animals entering the flock and even if the 95 percent fiducial limit of the expectation is taken into account this only reduces

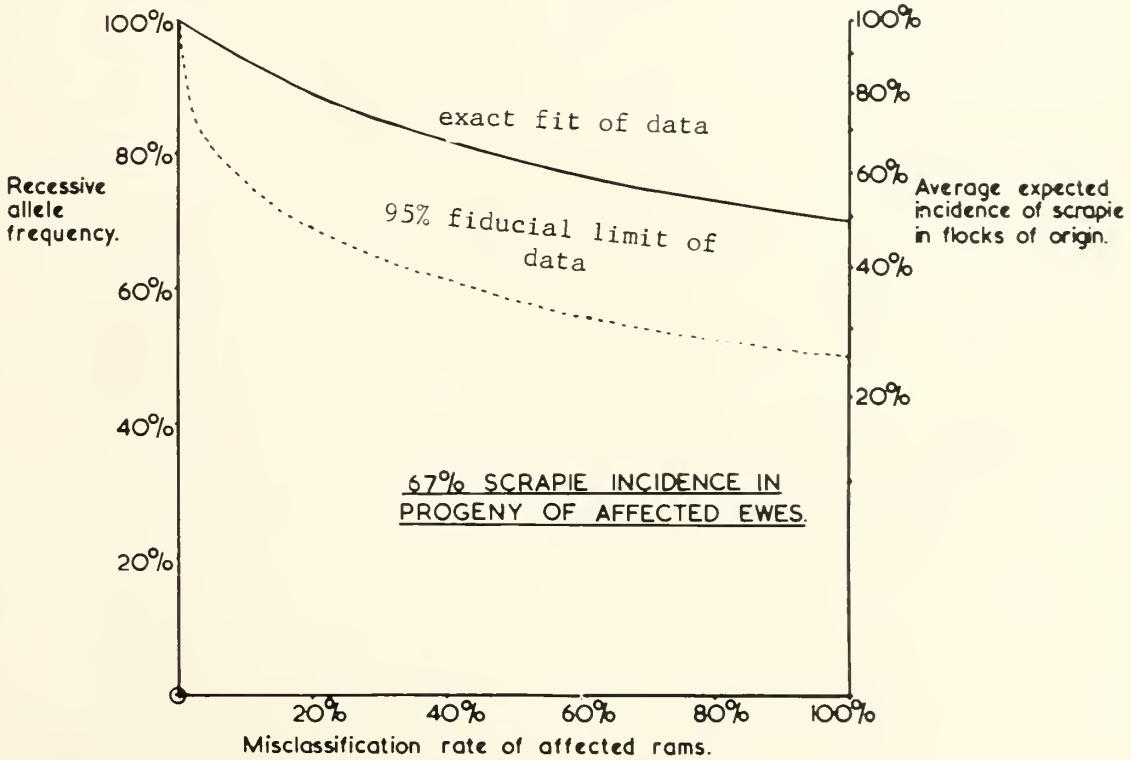


Figure 3.--The average expected incidence of scrapie on the affected farms if a recessive gene is assumed to have caused the 67% incidence in offspring of affected mothers

the predicted scrapie incidence to 25 percent, which is still wide of the observed value of about 10 percent. Making allowance for known biases - late developing cases, commercial culling, deaths unrecognised as scrapie - it is most unlikely that the actual incidence is as much as twice the observed incidence. This model which does not take the presence or absence of a pathogen into account, hardly fits the facts.

INCIDENCE IN PROGENY OF EXPERIMENTAL MATINGS

As with the foundation stock, the overall picture is again one of too much scrapie to reconcile with a recessive gene as the sole cause.

Every ram used--ten in all--whose progeny have attained appropriate ages, has had some of them affected. Four of these rams were deliberately chosen to be the "freest of the free" in terms of the criteria adopted for selecting OF stock and despite this they have produced a greater proportion of affected progeny when mated with affected ewes than would be expected even if they were all postulated to be heterozygotes ($P < 0.05$). The four rams have remained unaffected two dying at 6 and $6\frac{1}{2}$ years of age, the others being still alive at $6\frac{1}{2}$ and $8\frac{1}{2}$ years old. Details of these results are given in table 1 along with the results of affected x affected matings for which the incidence is very high indeed.

Most of the matings used in table 1 formed part of a larger series, comprising all four types of mating between OS and OF groups, including reciprocal crosses. The results for this larger series are summarised table 2.

Several points concerning the results in table 2 require explanation. The progeny groups contain approximately equal numbers of each sex but almost all the males were castrated. Every female offspring was given the opportunity to have two crops of lambs by an unrelated OF ram. There is no indication of a sex difference in scrapie incidence. Only two of the five OS rams are known to have developed scrapie; of the others, two are still unaffected, but the third unfortunately died early without signs of scrapie, though its surviving twin which was not bred did develop scrapie (only 5 percent of the progeny are from this third ram and their exclusion does not affect the trend of the results). The OF rams were three of those detailed above and of the OF ewes involved, none developed scrapie. The allocation of ewes to the various blocks was at random, each contributing successive progeny to the same block. However, in the case of OS ewes, there was a disproportion in their scrapie incidence between the two blocks: three-quarters of mothers of SF progeny developed scrapie, whereas only half of mothers of SS lambs did so (much of this difference resulted from ram infertility in the SS block matings in the first year). This disproportion is suggested as the reason for the lower incidence of scrapie in the SS animals than in the SF ones. When the incidence among dams of the SS block is adjusted to that of the SF block dams, the SS progeny incidence becomes 61 percent, instead of 39 percent if a linear proportionality is assumed - a procedure which may only have crude validity but with some justification from the previous results.

Table 1.--Scrapie incidence in progeny[1] of affected ewes mated with two types of ram

Mating	Progeny		
	n	Unaffected	Affected
4 unaffected OF rams x affected ewes.....	24	21%	79%
5 affected rams x affected ewes	21	5%	95%

[1]All unaffected animals $4\frac{1}{2}$ years old or older.

Table 2.--Scrapie incidence in OF and OS parents and progeny[1] of the four mating types

♂ \ ♀	OF	OS
OF	FF 10 ♀ parents 0% 3 ♂ parents 0% 27 Progeny 18%	SF 19 ♀ parents 75% 3 ♂ parents 0% 32 Progeny 69%
OS	FS 11 ♀ parents 0% 5 ♂ parents 40% 38 Progeny 10%	SS 24 ♀ parents 48% 5 ♂ parents 40% 46 Progeny 39%

[1]All unaffected animals $4\frac{1}{2}$ years old or older.

In the light of these remarks it is concluded from table 2 that the type of ram had little effect on the variation, the small differences due to ram-type are in the opposite direction to those expected on any solely genetic hypothesis. Instead, the main source of variation appears to stem from the type of ewe and this effect is consistent within progeny from both types of ram.

Another point about this experiment affecting possible interpretations must be stated. For reasons given earlier, the OF and OS ewes were loosely isolated from one another and the same applies to their progeny, FF and FS blocks being separate from SF and SS blocks at first. Since the total experiment was built up from 4 successive lamb crops, this initial separation lasted for 3 1/2 years for the earliest lamb crop and progressively shorter for successive crops.

SCRAPIE INCIDENCE IN STOCK BORN AND REARED IN MAXIMUM CONTACT WITH THE DISEASE

It has not been possible to assess what effect if any the above separation has on the results. In the early stages a numerically small experimental attempt was made to check the possibility of contact transmission so that this point would be covered. The small numbers - four ewes chosen at random from the OF group - were obligatory because of the extreme shortage of OF stock. These have been mated either to OF rams or FF rams and scrapie has now occurred in 40 percent or more of the immediate progeny of each of the four ewes, one of which has also developed the disease at 5 years old. The numbers in table 3 are too small to draw firm conclusions since the genetical equivalence of the two groups is uncertain. However, the average incidence here of 57 percent scrapie compared with 18 percent for broadly similar stock in partial isolation is sufficient cause to take the possibility of contact transmission seriously, the difference being significant ($P < 0.02$).

Table 3.--The incidence of scrapie in FF stock born and reared in different degrees of exposure to clinical cases of scrapie
All progeny 4 years old.

Rearing status	Affected	Unaffected
Maximum..... Contact.....	8[1]	6
Partial[2]..... Isolation.....	5	22

[1] No neuropathological examination possible for one animal.
[2] From Table 2.

GENERAL DISCUSSION

The need to postulate the involvement of a pathogen has been brought out in connection with the analysis of the separate aspects of the data and needs little further elaboration. Other types of work have also shown this necessity to allow for infectious or contagious transmission of scrapie. Briefly these are the oral transmissions of the disease in sheep, goats and mice (Pattison & Millson, 1961; Zlotnik & Rennie, 1962), transmission of scrapie from sheep to goats by contact (Stamp, 1962), contact transmission from mouse to mouse to be described later in this symposium, and the appearance of scrapie in free sheep placed on previously affected farms in Iceland. (Sigurdsson, 1954).

Two points arise from the present work which must be discussed, one being the question of what genetic control of susceptibility to the pathogen can be inferred from these experiments, the other being the need to explain the results of some matings, notably affected x affected, if a pathogen causes the disease: the latter point will be dealt with first.

There seems little doubt that when two affected Suffolk sheep are mated, their progeny almost always develop scrapie within four years. The same applies where one or both parents manifest the disease a year or two after the progeny are born, but with a disease of prolonged incubation, like scrapie, there is hardly any point in distinguishing this class from the former until more is known of the presumed infectious period. If a disease of very short incubation showed this property of always appearing in progeny of affected x affected matings then either the pathogen must be highly infectious and ubiquitous or some

mechanism for its non-genic transmission from the parents to the progeny would have to be postulated, such as maternal transmission. The necessity to infer the latter mode of transmission for scrapie in many Suffolks comes not from the affected x affected mating class, though they presumably have it, but rather from a comparison of reciprocal cross matings where only one parent has the disease. The evidence given points to the scrapie incidence in the progeny matching the mother's category. Furthermore some published information on natural scrapie can be interpreted the same way. Thus Parry's evidence on scrapie in twins shows two effects (1962, table 5): first affected ewes x unaffected rams produce 86 percent scrapie progeny ($n = 14$) whereas the reciprocal cross, where the male alone is affected, has only 26 percent affected progeny ($n = 27$). Moreover these same data when compared with a recessive gene only model have an excess of twin pairs both like their mother's category and a deficiency of pairs where only one member is affected ($P < 0.001$). This trend is not quite as marked in our own very limited group of twin pairs but the combined series show marked deviation towards similarity of mother and progeny and disagreement with the facts fitting a solely genetic hypothesis.

The postulate of non-genic maternal transmission of the pathogen seems the simplest explanation for the above twin effects, the high incidences in OS (dam affected) progeny, the SF and SS progeny and in progeny of affected x affected matings. However, it would be unwarranted to assume that the existence of maternal transmission in many Suffolks implies that this route will operate whatever the genetic constitution of the sheep - it may be absent in some other breeds or some lines of Suffolks and it is possible that one ram found by Draper and Parry (1962) confers on its offspring a genetic resistance to infection from the mother, since 18 progeny by affected dams remained free from scrapie for 3 years. However, the fact that maternal transmission of scrapie operates in at least some cases appears almost indisputable in view of the experimental demonstration of it by Gordon (1960).

Given the existence of a scrapie pathogen which some sheep do not encounter there is no need for elaborate hypotheses to account for the high proportion of unaffected animals which have some affected offspring. A high proportion of this class has been demonstrated in the present data, is also characteristic of some flocks especially flock A rams in the paper by Young et al. (this symposium) and much the same also appears to be true for the flocks detailed by Parry (1962).

Turning to the important question of what evidence of genetic control is present in our data, the outcome is inconclusive. Data such as those in table 1, where there is less scrapie, though not significantly so, in progeny of unaffected rams than from affected rams may indicate some degree of genetic control. However, some or all of the OF rams could be susceptible, though unaffected, because they had received no effective challenge with the scrapie agent, in which case their genetic contribution to the progeny would be no different from that of affected rams. Moreover, the high incidence in progeny of affected mothers lends itself to the suggestion that any genetic resistance which may be present in these data is largely overridden if the pathogen gets into the progeny at a very early stage via the mother.

REFERENCES

Draper, G. J. & Parry, H. B. (1962) Nature 195: 670
Gordon, W. S. (1960) U.S.D.A. Report ARS91 - 22
Parry, H. B. (1962) Heredity 17: 75
Pattison, I. & Millson G. C. (1961) J. Comp. Path. 71: 171
Sigurdsson, B. (1954) Brit. Vet. J. 110: 341
Stamp, J. T. (1962) Vet. Rec. 74: 357
Zlotnik, I. & Rennie, J. C. (1962) J. Comp. Path. 72: 360

Mr. Draper:

I agree with a great deal of Dr. Dickinson's comments obviously, particularly his comments about statistical significance and significant tests, and so on. But there is just one thing I am not absolutely clear about, and I think it is interesting, and it is not trivial. It is important. This data, as far as I can see, does get rid of the recessive hypothesis without a pathogen.

But I want to make the point--the point I did make yesterday--that I didn't exclude the possibility of a recessive gene which is conferring susceptibility, or if you like, needs a pathogen, and I want to know whether Dr. Dickinson thinks this is possible, or likely, or whatever.

Dr. Dickinson:

I have no data whatsoever to make this either more or less likely. This I am sure is disappointing to Dr. Gordon.

I don't think it is helpful at this stage to start putting up hypotheses, as I contend Parry did in a most elaborate embroidery sense in his Heredity paper to try to invent situations which might or might not occur. I want data. And in the face of his data, is there any evidence for anything here? My data says there is no evidence to encourage me to go on at this stage in terms of natural scrapie in the Suffolk breed to talk in terms of susceptibility or resistance. I would caution you in the interpretation of your one ram as being that particular interpretation. All it may be doing is preventing maternal transmission happening.

Let me explain what I mean in terms of a known model in mice. Gross' mouse leukemia, the last I heard of it--and I saw Dr. Gross on this point two years ago--is a maternally transmitted agent, but only in some kinds of mice. I don't remember the strains in which it happens, but I am going to refer to this question again. Some strains of mice prevent contagion from the mother, prevent transfer or transmission from the mother. But they are all susceptible. They can all be made to get it. So this is why I say, let's not bother talking about susceptibility or anything. Let's talk about our data, and see what it forces us to talk about. Susceptibility, resistance, all the rest of them. Many hypotheses can be held. It does nobody any good at this stage to do anything other than converse about them.

Dr. Gordon:

I would like to preface my remarks by reminding myself of a reference to golf in the Bible, something to this effect: And Ananias, after a bad lie, lay dead, and Sophia, his wife, following after, did the same, and halved the hole. Dr. Dickinson has dug a very big hole which I don't want to be halved with Mr. Parry. At the risk of being destroyed and thrown into this hole, I would like to draw attention to the slide of the histogram of the occurrence of scrapie in the off-free group.

This particular incident has a very strong resemblance to Grieg's experiment referred to in my first paper.

Dr. Dickinson:

Thank you. I forgot to bring this point out.

Dr. Gordon:

An important piece of evidence.

Dr. Dickinson:

I had meant to bring out this point.

Dr. Gordon:

It resembles Grieg's experiment very closely. Although you have excluded two of these sheep I would prefer to see them included.

Dr. Dickinson:

You can include them for this purpose.

Dr. Gordon:

They are late manifestations.

Dr. Dickinson:

Precisely.

Dr. Gordon:

I had the same experience when transmitting the disease to a large number of animals and contact controls became affected. These are late manifestations. It is very proper that they should be included and it is a piece of useful additional evidence on spread by contact.

Dr. Dickinson:

This is what I said about Parry's one case at $6\frac{1}{2}$ years old yesterday.

These three are all late. They are after the half life of a sheep. And so it is suggestive

that they got contagion sometime earlier. Whereas the majority which I suggest got contagion sometime prenatally, just developed it earlier. It is all very simple if looked at that way.

I would like to make my position completely clear on this. There are a number of geneticists in the audience, and if they remain silent could I take it that they find no particularly obvious flaws in this mode of approach to the data? I obviously can't ask them if they like the detailings of it. But I would like some commentary from them, if they are critical about this mode of approach. Because we have heard funny statements from geneticists about scrapie, who have not worked with scrapie.

Dr. Hutt:

To whom the cap fits, let him wear it. Dr. Dickinson, I hope you won't mind my saying that I found your presentation very difficult to follow, and I am afraid that quite a few of us might have been in as much difficulty as I.

As I understand it, and I hope you will comment on whether I am right or wrong, the analysis was made to determine the frequency of a single recessive gene by the present portion of animals affected as shown in your contingency table. You then go back and say the frequency could never have been that high in the four free flocks without the disease having occurred there.

This I think is a perfectly good technique. I think if I have understood it correctly, I have to confess that apart from that, and with that part I agreed, I would like to know what other conclusions you may have, if any.

Dr. Dickinson:

I concluded the same exactly, using the same logical sequence applied to flocks where scrapie was occurring and from which we had taken OS (dam-affected) offspring. Therefore, it gets rid of the question of whether the flock owners obscurred scrapie and we didn't see it. That is not a useful criticism there.

I again showed that you would expect more scrapie in terms of what we got on the farms of origin than was occurring and which we were buying.

Exactly the same sequence fits. But the criticism about reliability of flock owners and their integrity does not apply in that case.

Dr. Hutt:

This applies only if we are dealing with a gene which itself, a homozygous condition, causes.

Dr. Dickinson:

Yes.

Dr. Hutt:

Your plannings are not incompatible with the possibility that the susceptibility might be dependent upon a simple recessive gene or more than one of them which affect the susceptibility to some pathogen. Would you agree with that?

Dr. Dickinson:

I would agree with it since there is no evidence in the data on any genetic variation whatsoever. I mean it is not incompatible with many other things that I can suggest.

Dr. Hutt:

In other words, they could have a high incidence of genes--

Dr. Dickinson:

It is not incompatible with the fact that there is no genetic variation for susceptibility or resistance. That is what I mean.

Dr. Hutt:

It is not incompatible with the hypothesis that on the free farms there were some sheep genetically susceptible, but which had not therefore been exposed, and to put them into a place where they are exposed that susceptibility is revealed.

Dr. Dickinson:

It is not incompatible with that, but it is also not incompatible with the fact that every Suffolk sheep, barring these particular important ones or this particular important one and its progeny, pretty well every Suffolk sheep is susceptible. And all that in fact is happening is that all Suffolk sheep go through a phase in their early life where they are relatively more susceptible than in their later life. You only therefore get a high incidence where they are bound to get exposed to it in their early life, that is from their mothers. You can see the point I am trying to make here. There is no evidence that I see in my data, nor in Parry's, except for this one ram, to indicate genetic variation.

There is familial relationship because of the maternal transmission and Parry's data are familial relationships.

I don't want to be made to say anything here unnecessarily. Scrapie plays so many tricks that I just don't think we should prejudice the issue at this stage unless we have data to say, "this data is forcing me in that direction." I say this data is forcing me away from the direction you propose and leaves me just where we started.

Dr. Hutt:

I can see that you have pretty good evidence that the incidence can be raised as a result of maternal transmission. Did I understand you correctly to say just now that in your opinion 100 percent of the Suffolk sheep are susceptible?

Dr. Dickinson:

No, I didn't say that. I am saying on the basis of a large sample it is a tenable hypothesis that with this one exception--the only piece of evidence we have against it, one animal--the evidence, apart from that one, is tenable with the suggestion that there is no genetic variation affecting susceptibility. You are trying to get me to say that there is variation which is genetically controlled. I was objecting to this. Its alternative is equally acceptable as things stand.

Dr. Hutt:

I wasn't trying to get you to say anything. I was trying to find out what you think.

Dr. Dickinson:

Thank you. Sorry.

Dr. Wells:

I visited Dr. Dickinson at Moredun earlier this month.

Therefore, I do not deny his accomplishments at all. Neither am I going to put my foot in the trap and ask the magic question which is going to put justification for my question back on my rather weary shoulder.

As I understand you, Dr. Dickinson, you said I am not going to be quoted as having said that we have no proof that scrapie can be spread from sheep to sheep by contact in the field. I tried to take it down as quickly as I could.

I also am not going to be quoted as having said that we have no proof that scrapie can be spread from sheep to sheep by contact in the field.

Dr. Dickinson:

That is perfectly correct.

Dr. Wells:

Without putting my foot in the trap, could you make the same statement in the positive sense and say that you can be quoted?

Dr. Dickinson:

Yes, I am perfectly willing to make such a statement. It is like a situation of finding your way somewhere, and you follow a signpost. And the situation, as I see it, is that all the signposts point in the same direction, and they all say to me, there is a pathogen which is not highly contagious but which is, maybe quite often, perhaps only just infrequently, contagious in sheep in a situation with natural scrapie in Suffolks. But I don't want to go outside of Suffolks, and I don't want to go outside Moredun.

So all I am saying here is that because of the nature of this agent, pretty well all our experiments have been done where there is an abundance of scrapie agent. I don't think that is really relevant to the issue, because we can't--I must speak carefully--we have had the utmost difficulty persuading Suffolk sheep to develop scrapie with Cheviot agent by coaxing, persuasion, or shock.

We sheared some Suffolk sheep throughout one winter. They were never allowed to grow more than a crew cut--and it was a cold winter. They probably ate 8 times as much as the normal sheep eats. They certainly didn't produce scrapie earlier or in any greater density.

We tried to coax animals in all sorts of ways to get scrapie. We challenged groups of Suffolks on two or three occasions with our Cheviot agent, which Moredun and all the area around has in abundance. We didn't produce any scrapie within two years in the Suffolk sheep. But, if I came and did these experiments again in Canada, I perhaps wouldn't find these results.

I want to be honest with everybody. I have worked with scrapie long enough to know that it is a brute, and so you have to take the most

critical possible view of your data. You have to say that a particular result is highly significant and you have to laugh at it. And you have to say, let's try and beat that result down however we possibly can.

I say this--and this is my answer to Parry in his opening remarks yesterday--we have not been publishing our data for two reasons. The data take a long time to come in. You have to keep sheep for years before you can draw conclusions of the kinds we have been drawing.

The conclusions that we have drawn and which we have been perfectly willing to discuss in private with Suffolk breeders are important conclusions to many people. Many people have a lot of money at stake on the solution of the scrapie problem in sheep, and we think it utterly unwarranted to put data that are half-baked in front of people and not tell them how half-baked the data are.

So this is our justification. Mr. Parry criticized me yesterday for having seen his paper before it was in press in Heredity. It was sent to me; he knew it had been sent to me, and this is my criticism. I was not asked to referee that paper. I sent it back to the editor saying it had no effect on the experiments that we were conducting.

If I had been asked to referee the paper, I would have produced detailed criticisms of it, and I would have recommended that it should not be published in its current form.

We are trying to be responsible here to people who have sheep and reputations at stake and who like their sheep very much and who may have them eradicated or may lose them en masse because of scrapie. So you can see why I am trying to be cautious and why I am trying to knock every bit of my evidence to pieces.

Dr. Wells:

Doctor, I appreciate this. This approach is of value to us.

You said specifically--and I don't want to make an argument of this--but want to know if you would change one word and put your statement in the positive sense, rather than negative.

Dr. Dickinson:

I will put it in the sense that every indication I see in the data points to the fact that scrapie can occur by contact, but that usually, in our experience, it does not occur by contact except by the special route from the mother.

Dr. Gordon:

I would like to concur with that statement.

Dr. Wells:

I think this statement satisfies me.

You see, you said you would not be quoted as having said that you have no positive proof that scrapie can be spread from sheep to sheep by contact. This left us completely up in the air.

I am quite happy with your amended statement.

Dr. Dickinson:

I wanted to make it absolutely clear to the farming press that I am not prepared to be misquoted and botched on this issue. I am not prepared to back up any nonsense statements in the farming press such as we have had to suffer for quite a long time.

Dr. Wells:

I am quite happy with the amended statement which can be quoted.

Dr. Gordon:

I faced the same question on Monday that you have been trying to answer today. I said on the evidence that I had presented that the possibility of spread by contact could not be excluded, and for some very substantial reasons. I know Greig experienced this; I experienced it; it has been recorded in goats in France and at Moredun; Pattison has observed it in mice and now there is good evidence in sheep.

On the first occasion, I might accept it as a coincidence; on the second occasion I become skeptical about it being coincidence but, when it comes to a third, fourth, fifth and sixth occasion, I have to accept it as evidence of spread by contact. Like Dr. Dickinson, however, I would like to say that this does not appear to be the most important method of spread, there is obviously some linear spread in family lines from the affected mother to her offspring, especially if the ram has contributed susceptibility to that offspring.

Dr. Burger:

You mentioned the vertical spread, and you mentioned, I think, in the beginning of your presentation the impossibility to get rid of that agent when it is around somewhere.

I wonder, how can you tell Compton controls are unaffected?

Dr. Dickinson:

This is the difficulty. I don't really want to enlarge much on it. Let me exemplify one type of difficulty. One has to try therefore to keep little groups of sheep, breeding groups, carefully balanced genetically in isolation. That is you ask a breeder if he will keep such a group. You have to ask a breeder who doesn't keep sheep, perhaps he keeps cows but in that case, he doesn't have fences, usually, to keep sheep in.

You dare not ask him to have more than about a dozen because--say he has a hundred. One case of scrapie crops up, and you wipe a hundred out.

You have to do it on a large enough scale, but on a small enough scale in each place to, in the end of the day, have some groups left which didn't get a case, but also to have some groups left which have susceptible sheep in them.

As Dr. Stamp said, there is no point at all--if susceptibility means anything--in proving that resistant sheep cannot get scrapie by contact. This will prove of no value for the real problem.

Dr. Gordon:

I have to answer for Compton. The method adopted there is briefly illustrated in the slides that I showed in my third paper yesterday. We are fortunate in having isolation buildings on an island site surrounded by roads within which we have never had a case of scrapie and where we breed scrapie-free sheep shepherded by a staff that never handle affected animals.

In the purchase of my 1,000 Herdwick ewes and 43 rams, 140 Dorset Down ewes and 7 rams, care was taken to ensure that scrapie was unknown on farms of origin. I have already worked with sheep of similar origin and no spontaneous case of scrapie has ever been found. Further, in the case of the Herdwicks the rams and ewes were all over 4 1/2 years old and the majority was beyond the age at which scrapie might occur. They have now produced two good crops of lambs and I am astonished that these old sheep have produced so well because some of them are 14 years old. I now have 50 flocks, 43 of which are Herdwicks and 7, Dorset Down.

When we come to test either offspring or parents for susceptibility, they are removed from isolation to pasture about a mile distant and shepherded by separate staff. When these animals are challenged, I contend that it is the first time the scrapie agent has been introduced to them.

With these facilities and this kind of sheep, I am able to undertake experiments to determine if my hypothesis is correct that a heritable genetic constitution determines susceptibility and that there is an environmental factor represented by the transmissible agent.

Before anyone else does it for me, I should point out that one disturbing piece of evidence is the resistance to scrapie on challenge in 19 of 52 offspring from parents that were both susceptible. This is discussed in my third paper. It is puzzling and I thank Dr. Dickinson for this afternoon's talk on genetics because he has probably taught me more than I could learn in a long time out of textbooks on genetics. I also accept this warning about the care necessary in scrutinising results before they are accepted as evidence and I will try to avoid the need for him to dig a hole for me and my evidence at a later date.

Dr. Goode:

Dr. Gordon, I am sure that all of us certainly envy you for having the facilities that you do over there. I know of no other place where we have them in the same numbers, with the same security, yet the same flexibility as you have.

Mr. Parry had indicated to me earlier that he wanted to speak last to discuss this. The time has arrived for that.

Mr. Parry

The ones in contact, were they in contact with natural Suffolk cases?

Dr. Dickinson:

Yes, there were always some natural cases present.

Mr. Parry:

Have they had any of the post inoculation? Are the animals in contact with the natural cases of Suffolk?

Dr. Dickinson:

Dr. Stamp dealt with the situation that you can start distinguishing on pathological criteria between the two agents we are dominantly using. This helps us to cover the kind of point you are making.

Even if not in contact with inoculated animals, I would say this is not really the issue. We have to assume, at this stage, that they are potentially in contact with the Cheviot agent.

Mr. Parry:

Which is being transferred through a sheep or goat by inoculation?

Dr. Dickinson:

They were constantly in contact with natural Suffolk scrapie.

Mr. Parry:

But any others? Passaged strains of scrapie?

Dr. Dickinson:

They were never in contact with Suffolk agent, passaged. They are inevitably in contact with an environment oozing with the Cheviot agent. I call it SSBP/1 agent, which is now in its 24th passage mainly in Cheviot sheep. I think it is true to say that they have never had immediate contact with any challenged animal of any sort whatsoever. But I think this is a fine point of difference only from the fact that the fields which they are in have carried that kind of animal, et cetera.

Mr. Parry:

I accept your point about this, and--

Dr. Dickinson

This is what I say. If you do this in Canada you may get different results.

Mr. Parry:

The point is, you see, that I have some results which may have a bearing on what you were saying. I didn't have a chance to present them yesterday in the hurly-burly.

The Heredity paper was an initial one. After all it represented 10 years' work and it is just as well to put down on paper what you think as a working hypothesis to see whether it is going to be right or not. I would say in passing that the twin table was purely supplementary, as you know, to the main thesis.

Dr. Dickinson:

It should have been analyzed. You drew conclusions from it.

Mr. Parry:

I merely said it was possibly consistent.

Dr. Dickinson:

Shall I read what you wrote? You are trying to detract from the Heredity paper. You were saying things strongly--

Mr Parry:

The number of sets in each category is small, but the results show a clear trend which is consistent with the genetic--

Dr. Dickinson:

It showed a clear trend of one in a thousand against it. I am sorry.

Mr. Parry:

I shall have to leave that to you mathematicians. The point is this was consistent with the hypothesis--

Dr. Dickinson:

It isn't. Before you published it I criticized you twice, severely, on the logic in it, and yet you went and published it.

Mr. Parry:

I am an amateur geneticist, as you all will have gathered. We sent this to the leading genetic journal in England, and I thought if it got past the editorial board at least it would be reasonable. I didn't send this to a veterinary journal. Otherwise Dr. Dickinson's strictures would be quite justified. I did endeavor to measure up.

As a result of that we laid down experimental breeding groups at home which are just coming to fruition. I have also on the same farm

post-inoculation cases, and I am getting very surprised because I have had three triplets out of an unaffected Dorset Horn ewe by an affected ram--if I may be permitted still to call my black ram such--going down. Last week there were signs that another set of triplets of the other backcross that are going down, and this is very worrying because this is not what I have disclosed so far from the field data. I am looking for an explanation.

You have presented data today which I know you have presented in my presence before. But like Dr. Hutt, or even more than Dr. Hutt, I find it difficult to take all this in quickly in a lecture, and we have been waiting for publication. This is why we wrote to you.

The point is, it may be that our results--it looks as if they are going to be similar to yours. But we are faced with the position that as far as I can ascertain in the field, in Suffolks, the data I showed you yesterday afternoon have not disclosed any spread of the disease.

Dr. Dickinson:

It could all be evidence of spread for all you know, couldn't it?

Mr. Parry:

I am only talking about clinical cases. I am unable to demonstrate the agent very effectively.

Dr. Dickinson:

Can I answer this point, about my presentation being complicated? This is irrelevant to the issue. I had two basic criticism of your presentation. First, I said it was a circular argument.

Secondly, I said you have your data from a culled population. It is not that mine is complicated, but that yours doesn't show what it says it shows. It is not a valid analysis of your data. This is where we started. This is complicated. Genetics is. I am sorry there are not more geneticists here.

In your papers, your conclusions, such as the twin one, you clearly stated that it shows it is consistent. It doesn't. We can't have this.

Mr. Parry:

We live and learn.

However, the point is that we endeavored in the Nature paper, with the help of Mr. Draper, to clarify and put on a firmer statistical foundation the gaps which we knew existed in the Heredity paper.

Mr. Chairman, I don't think there is any point in discussing this any further. Where possible, we are checking back and my experimental data will be coming up. It will come to fruition in the next 2 or 3 years.

The initial results obviously are confusing to me with regard to my results in the field. I don't know what the answer is. But there is a conflict. And I think Dr. Dickinson's paper, for which I thank him for putting this information before us, will maybe help us to clarify this position.

Dr. Goode:

I think we all appreciate the fact that there is other work developing around this area.

The data presented today will be taken into consideration and reviewed and evaluated with regards to the new data developed.

Dr. Mulhern:

Dr. Dickinson, unlike Dr. Wells, I want to see if I understand in a little way the situation as it is presented. If I understand this correctly, you are stating that the spread--that there is a transmissible agent that is generally transmitted from the maternal transmission, and that there is, you believe, also a contagion which is occasionally responsible for the disease.

Dr. Dickinson:

In our data?

Dr. Mulhern:

Yes. And that your data also suggest that there is reason to believe that there is a degree of spread by contact.

Dr Dickinson:

I think this is the simplest logical explanation, yes.

Dr. Mulhern:

Therefore, if I understand you correctly, you are saying that there is a difference between what you believe and the recessive gene hypothesis, and that from your standpoint it isn't a degree of resistance and susceptibility, but that they may all be capable of developing

the disease. It is just a matter of whether they are exposed to the transmissible agent, except in that one ram which may produce interference.

Dr. Dickinson:

Broadly, yes, this is true.

I would like to comment on how our data differ from the broad summary of the United States eradication program data tomorrow morning, because there are differences which people will probably go home and start recognizing and say, "My data doesn't fit yours." But this is a function of the culling introduced by the eradication system.

Dr. Stamp:

Am I not right in thinking that what you have just confirmed with Dr. Mulhern only goes for your own data?

Dr. Dickinson:

I could never say more than that.

Dr. Stamp:

I must stress that you can not automatically transfer this or that from our data and then say this happens in the field. We do say this may happen, or that may happen in the field.

I would not like you to go away with the idea that only maternal transmission can happen, and that contact transmission doesn't happen. All we think is that maternal transmission occurs and that contact transmission occurs. The extent of either of these things in the field we do not know.

All our information is from our own experiments. We are scientists and we are not administrators. It is up to you in the USDA to draw your own conclusions from these data.

Dr. Goode:

I was wanting to know whether the Suffolks have been referred to here only because of the fact that they were more or less a part of the experimental model; they were available; they had certain characteristics that did lend themselves at the time of the examinations that they received.

Dr. Stamp:

We were accustomed to these Suffolk sheep. We knew them, and they were available. And therefore we used them. This is the answer to your question.

Dr. Hutt:

I would like to get one point clear for my own benefit.

As I understood the presentation in a varying proportion of cases when sheep are inoculated some of them don't take. Some cannot be inoculated. Is that right?

Dr. Dickinson:

I will be dealing with this in, I think, a 3-minute paper shortly. The presentation will just be condensed to one table.

Could I answer that later?

Dr. Hutt:

I would like to know how you account for those. How do you explain that they don't all come down?

Dr. Dickinson

It looks very much as if they are resistant.

Dr. Hutt:

What way?

Dr. Dickinson:

I would think the simplest explanation is not that they are resistant because by chance they didn't get an infective challenge. I think the simplest explanation is that they are genetically resistant to that agent.

Sorry: that is the point; to the one agent, SSBP/1.

Dr. Hunt

This is precisely the point I want to bring out; that there can be genetic differences in susceptibility and resistance, even though it is not the gene that causes the specific disease.

Dr. Dickinson:

This is perfectly true.

And Dr. Gordon's presentation the other day specifically to this point, specifically with SSBP/1/24, proved this to my mind, that there is a considerable degree of genetic control of that particular character.

My own data are identical with his. They are not as far advanced and on a smaller scale.

In terms of that agent it seems, superficially, that you have to talk in terms of resistance or

susceptibility. When you come to the mouse paper this afternoon you might be qualifying what you said, and so will I.

Dr. Stamp:

The point I would like to make is that you have to remember that when we are using Suffolk sheep we have to limit the time period during which we can say these sheep went down to the challenge inoculum. A stage is reached in the experimental procedure with sheep beginning to go down with scrapie when one doesn't know whether they are going down with the natural disease or the experimental disease.

In my paper this morning I was very careful to say "sheep that we think have gone down following the inoculum." I was careful to emphasize this difficulty.

Mr. Parry:

May I comment on Dr. Mulhern's question and Dr. Stamp's answer.

This is only relevant to the situation, the experimental situation, just as my home data is only relevant to my situation.

What I would like to draw your attention to is that out of the flocks in our Health Scheme, if all goes well, we shall get data from a group of field flocks and under field conditions. This perhaps will help to clarify some of the difficulties.

I would like to stress this because I believe that accurate field data under the circumstances I outlined yesterday, may have a real part to play in unraveling this problem.

Dr. Dickinson:

I agree with that remark. One just has to be more cautious with field data.

Mr. Yohe:

For the clarity of the record, come the end of this week some of us cannot be cagey. We have to sit down and decide what we are going to recommend. You scientists are certainly in better position than some of us. And so I would like to ask at this point:

Dr. Stamp, it would be your belief, or if you were in our position and had to make a premise, your premise would be that scrapie can be transmitted by contact.

Dr. Stamp:

I am a scientist. I find this question very difficult to answer.

Mr. Yohe:

At this point--not tomorrow. You may change your mind.

Dr. Stamp:

There is good evidence that would suggest contagion. There is evidence which strongly suggests it.

Mr. Yohe:

Would you agree with that, Dr. Gordon?

Dr. Gordon:

I have already said the possibility of spread by contact cannot be excluded--

Mr. Yohe:

Let's get this in the positive mode. We must base our recommendations, which the USDA may or may not take, and they must base theirs upon a premise that it can or cannot be. I am asking you at this point if you were in that position what premise would you take?

Dr. Gordon:

I personally am satisfied that the experiences that I and others have had enable me to conclude that spread by contact is a definite possibility and can occur. I merely expressed it the other way round, incidents per se have been accepted as evidence.

Mr. Yohe:

I understand.

Dr. Dickinson:

Would you like me to be positive?

I think the issue that you have to decide is slightly different than that. I am willing to go as far as saying that it seems to me most reasonable, because of the pointers in that direction, that scrapie can occur by contagion. It is up to you to decide whether ours are data that are relevant to the populations which you are making judgments about; and this is not our province, this particular province.

Dr. Goode:

Dr. Anderson has asked me to make an announcement regarding tomorrow's meeting. He wanted it brought out that each and everyone here is certainly invited. It is hoped that you all will be able to attend tomorrow's meeting.

Dr. Dickinson:

I have been asked one point by one of our Canadian friends concerning a misunderstanding that might have occurred.

I think we have hinted, and we feel in no position to do more than this, that some bits of the data do suggest to us--field data, and other experimental data, besides the ones that I have presented to you--that on some occasions, rams can, in fact, be very destructive in the sense that many of their progeny go down as though introducing that ram into a flock is a fatal thing to do.

We don't think it is anything more than suggestive. But we think there is something in this worth investigating; and since it is important from the point of view of those who have to make up their minds tomorrow afternoon, it is something that maybe we ought to share with you at this very hesitant and preliminary stage. This will be presented as a table tomorrow.

Paper No. 29

SCRAPIE: EXPERIMENTS INVOLVING MATERNAL TRANSMISSION IN SHEEP

A. G. Dickinson,[1] G. B. Young,[1] and C. C. Renwick[2]
(Presented by A. G. Dickinson)

A number of years ago when the probable importance of maternal transmission in Suffolk scrapie was becoming evident in the results of breeding experiments, a number of small exploratory experiments were started to eliminate or clarify some of the possible maternal routes. A brief report of this work is given here and one purpose which it will serve is to demonstrate the tactical difficulties met in experiments of this sort. Many more questions have emerged from these experiments than have been answered.

A COMPARISON OF NATURALLY FED AND BOTTLE-FED LAMBS FROM DALESBRED AND SWALEDALE EWES AFFECTED WITH NATURAL SCRAPIE

By analogy with the mouse mammary tumor agent which is passed in the milk it was of interest to determine whether scrapie transmission might be confined to the ewes milk. Unfortunately a third of the lambs died for unrelated reasons before 9 months old bringing the number down to those shown below (table 1) which are too few to establish statistically any but the most marked differences. However the results do show that the appearance of scrapie before 3 years old is not prevented by artificial rearing.

Table 1.--Incidence of scrapie in progeny of ewes affected with natural scrapie (Dalesbred and Swaledale)

Item	Affected	Unaffected by $3\frac{1}{2}$ yrs. old
Reared on ewe	6	2
Bottle fed	2	5

[1]Animal Breeding Research Organization, Edinburgh, Scotland.
[2]Clinical Investigation Department, Animal Diseases Research Association, Moredun Institute, Edinburgh, Scotland.

Apart from the main purpose, these results further underline the role of material transmission because the in-lamb affected ewes were purchased as a random group, having been mated with a variety of unknown rams and the scrapie incidence in the progeny shown in table 1 is high--too high to accord satisfactorily with a recessive-gene-only hypothesis, since they would indicate an allele frequency of 50 percent in the population from which these ewes came. This does not tally with the field incidence especially in view of the alleged extreme rarity of affected males in these breeds.

EXPERIMENTS INVOLVING EGG TRANSFER

At first glance it should be easy to prove that maternal transmission of the natural disease can take place by using egg transfer techniques. The requirements would be a breeding line of sheep which could develop scrapie on encountering the agent but which did not do so when bred in isolation--these would contribute eggs. As recipients, ewes whose natural progeny could be positively distinguished from any transferred embryos--either by use of a different breed or by blood typing--and which would develop natural scrapie, preferably during the resulting pregnancy. It is now feasible to meet these requirements in the case of recipient animals. However, it is at present impossible to fulfill the requirements for donor stock. Apart from the intrinsic technical difficulties of egg transfer, some other snags have come to light in our early attempts to tackle this problem. Since scrapie is such a resistant agent, it is impossible to organise surgical facilities which exclude the risk of exposing the susceptible donor ewes to the disease if affected sheep are among those undergoing operations. It is worth noting that the first case of scrapie in our so-called "free" Suffolk line was the only animal which had undergone operation as an egg donor, two years before the clinical signs developed at $4\frac{1}{2}$ years old.

Apart from certain theoretical reservations these latter difficulties can be avoided by using two unaffected susceptible breeds as donor and recipient, conducting the surgical transfer using personel and facilities unconnected with scrapie research and later challenging the recipient ewe by inoculation but leaving the donor unchallenged to produce natural control progency at the following oestrus. This is one of the combinations we have used and we are indebted to Dr. J. L. Hancock for conducting the operations. The lambs are the genetic offspring of a free-line Suffolk ewe, now almost 10 years old and still unaffected, and a free-line Suffolk ram which died unaffected at 6 years old. The transferred egg was placed in a Border Leicester ewe, (herself the progency of an affected sire) and she was given a 5 ml. S/C dose of 10 percent SSBP/1/21 agent 17 days after the transfer operation. This ewe first showed clinical scrapie at about the time the lamb was weaned. The lamb developed scrapie at 4 years old and this was confirmed histologically: the clinical syndrome in this animal when it had to be killed was advanced incoordination but there was no loss of wool to indicate any pruritis. A control natural-born lamb, full sib to the transferred one, has also been reared. The control, which came from the first ovulation after the egg recovery operation, is now almost 5 years old and has not developed scrapie.

Though suggestive, no firm conclusions can be drawn from this isolated experiment. Until more appropriate stock are available in large numbers further egg transplanting experiments are not justified.

TRANSMISSION TO PROGENY OF EWES INOCULATED WITH SCRAPIE

Scrapie appeared in about 7 percent of Cheviot lambs at an atypical age (7-18 months) following challenge of their mothers with scrapie agent shortly after mating: this was reported by Dr. Gordon in 1959. We have undertaken a similar experiment with a related strain of agent but in a different breed combination.

Five Border Leicester ewes were mated with an Oxford Down ram and those which returned to service at the next oestrus were mated with a Suffolk ram, which later developed natural scrapie. The ewes were challenged with scrapie one to two weeks after first service but before any return to the Suffolk ram. The inoculation given subcutaneously,

was 5 ml. of 10 percent scrapie brain suspension from a Border Leicester ewe used for passage of the SSBP/1/21 agent. As in Dr. Gordon's case scrapie again appeared without direct challenge of the progeny: 2 out of 4 of them developed the disease at the unusual ages of 8 and 13 months, the first being a Leicester x Oxford cross and the later one a Leicester x Suffolk cross. These ages are unusual in the sense that with the possible exception of some North Indian sheep (Zlotnik and Katiyar, 1961), natural scrapie is never seen before 18 months old.

It is, of course, possible that natural scrapie was simply hastened by the treatment to which the animal's mother had been subjected. Alternatively, induced scrapie may have been delayed, possibly due to extreme dilution of the agent reaching the embryo. The suggestion of delay comes from the fact that in direct challenge of lambs at birth with this agent scrapie starts occurring from four months old. Whatever ultimate explanation of the mechanism emerges, there is no doubt that challenge of the mother produces scrapie in the offspring which would not otherwise have appeared at least at these ages. Figure 1 summarises the contrast between lambs acquiring scrapie due to maternal challenge and those in which the disease has a different origin.

This situation prompts the question "Why does not scrapie appear at these early ages in lambs of mothers naturally affected with the disease?" One explanation would be that there are a variety of scrapie agents, presumably differing genetically, and that no agent is at large in the field which gives scrapie in less than two years when transmitted naturally. The speculative reason for this restricted scope of agents in the field is quite simple: natural selection on the agent would preclude the establishment of strains with shorter incubation period that the usual age at first lamb, two years, if maternal transmission were the predominant route. In fact the self elimination of a short incubation period agent in the field may have been observed: scrapie was unmistakably found to be transmissible to sheep some years ago when an accidental challenge of about 18,000 animals occurred by giving them louping ill vaccine contaminated with scrapie. About 10 percent of the animals given the vaccine developed scrapie (Greig, 1950) and we have several reports that lambs from these sheep were also affected but the disease did not seem to have continued into any further generations.

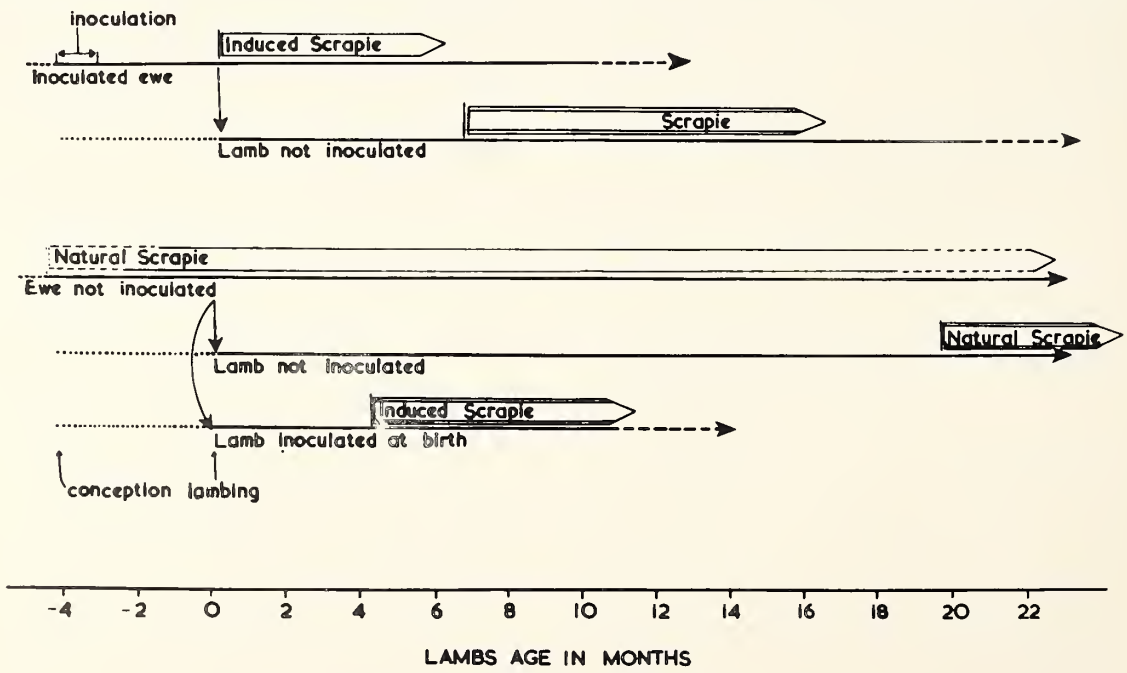


Figure 1.--The relative ages at which sheep develop scrapie according to the route by which they acquire it

So far as maternal transmission is concerned an important question for investigation is whether there is any genetic control of this route. One example of this type of control is known affecting leukemia in mice (Gross, 1961): in some mouse strains maternal transmission is the main route of transfer to the young, in other genotypes no agent apparently passes by this means. Although all, or almost all, goats are susceptible to injections with scrapie agent and 4 out of 6 goats of three breeds developed scrapie by contact with sheep (Stamp; this symposium), nevertheless, there have been no observations to suggest that scrapie can be passed maternally in goats. This may be the main factor to account for the apparent lack of scrapie as an endemic disease of goats even though sheep and goats graze together in some countries and scrapie has been reported in a goat in France grazing with affected sheep.

The complication now introduced into experiments to investigate maternal transmission, for which mice could be ideal material, is that contact transmission has been shown to occur from mouse to mouse. Experiments with a few female Moredun mice injected intraperitioneally with scrapie at about the time of mating have yielded one probable case of scrapie out of 4 progeny examined histologically at 7 months old. However, large scale breeding work using many strains and crosses, employing female mice which had been injected with ME 7 scrapie agent when they were weaned, has so far produced no clinical scrapie by one year old, after which age, if not before, it will be difficult to distinguish any maternal transmission from contagion.

REFERENCES

GREIG, J. R. (1950). J. comp. Path. 60: 263.
GROSS, L. (1961). Proc. Soc. Exp. Biol. Med. 107: 90.
ZLOTNIK, I. and KATIYAR, R. D. (1961). Vet. Rec. 73: 543.

DISCUSSION

Dr. Gordon:

One point I should like to make. In my second experiment that Dr. Dickinson referred to, and I have mentioned this and I will see that it gets into the notes (see figure 6 in addendum to my 3d paper), we allowed the offspring to reach approximately 19 months of age. Up to that time only three developed scrapie, possibly by maternal transmission.

Now, we challenged the surviving offspring when they were 19 months of age, and there were still susceptible animals amongst that population.

So this I think is a very important point. As with the possibility of spread by contact to susceptible animals, so with spread by--shall we call it maternal transmission--some susceptible animals do not develop infection within a reasonably short time. If these susceptible animals are left in a scrapie environment, my worry would be that some might pick up infection at a later date and fall into the category of late manifestations, possibly obscuring the fact that they were contact infections of some kind.

Dr. Dickinson:

I couldn't agree more that the control of spread by these two routes may be entirely different.

Dr. Gordon:

There is one other point I should like to mention, because it illustrates the great difficulty of work with sheep of unpredictable susceptibility. We undertook an experiment involving 78 North Scotland Cheviot ewes. They were dividied into two groups, one of which was inoculated with scrapie. The lambs from inoculated ewes were twinned on to noninoculated ewes at birth and the lambs from noninoculated ewes on to inoculated ewes. The high hope was that it might throw more light on the possibility of maternal transmission. After all the work involved,

the experiment yielded nothing because only three ewes developed scrapie. This is the kind of frustration one faces in experiments with sheep of unpredictable susceptibility. That is why I am attempting to obtain experimental animals of known susceptibility by breeding at the two ends of the susceptibility spectrum, in the hope that two diametrically opposed populations can be obtained, one as near as possible a hundred percent susceptible, the other one hundred percent resistant. I think we are making good progress in this direction.

Here, I may say that a susceptible ram can cause a dramatic rise in the susceptibility of his offspring and, conversely, a resistant ram can dramatically depress the susceptibility of his offspring. This has a resemblance to Mr. Parry's "whiter than white" ram, whose offspring don't take scrapie but his explanation of this differs from mine. In the context of my hypothesis, this ram is homozygous resistant and confers resistance on all his offspring irrespective of the susceptibility of the mother. Here I may run the risk of being cast into the hole dug for Mr. Parry! Please don't retaliate at the moment, Dr. Dickinson; this is only a working hypothesis and I will try to be careful in obtaining good evidence to avoid letting you get as big a grip on me as you have on some other people.

Dr. Kurland:

With respect to your first slide, where you showed the number of offspring that were bred by mothers, were any of those offspring male or all female?

Dr. Dickinson:

I can't give you the details there. I know at least one of the affected animals is a male, but I can't guarantee if it was a wether or not. We do know that natural scrapie has in our experimental work occurred in Swalesdale and Dalesbred males. This just does not match up with the weird trend alleged by breeders in the field.

Dr. Anderson:

I would like to refer back to the same slide that Dr. Kurland referred to, the one on the incidence of scrapie and progeny of affected ewes. Particularly that part dealing with the bottle-fed lamb.

Were any of those lambs isolated in apparently scrapie-free quarters?

Dr. Dickinson:

No. It is the old argument again, that experiment is full of bullet holes already. The answer is, "No." I don't know where the cow's milk came from that they were fed on. This was sometime ago. I thought it was just worth dropping this one in, because it shows the one or two little trivial points that come out.

I couldn't say anything more in these terms when you get down to looking at it closely. It means very little.

GENETICAL CONTROL OF SUSCEPTIBILITY TO EXPERIMENTAL CHALLENGE WITH SCRAPIE IN CHEVIOT SHEEP

A. G. Dickinson, J. T. Stamp, C. C. Renwick, and W. Smith [1]
(Presented by A. G. Dickinson)

In the experimental transmission of scrapie to sheep, animals of the Cheviot breed have been generally used, the transmissible factor being the SSBP/1 agent first isolated by Wilson et al. (1950) which has now been passed 24 times, mainly through Cheviot sheep. The incidence of experimental scrapie in inoculated sheep has varied considerably (Stamp, 1958), but in Cheviots it rarely exceeds 50 percent and is frequently about 30 percent, but it can be less. In samples of other breeds of sheep the incidence ranged from 78 percent to 0 percent (Gordon 1960), but no other breed of sheep has been so extensively challenged as has the Cheviot.

This evidence of the sheep's varying susceptibility to experimental scrapie suggests that there may be some genetic factor or factors which control the occurrence or non-occurrence of scrapie in inoculated animals. In consequence, a selection experiment was initiated with the object of producing Cheviot sheep of high and low susceptibility to experimental challenge with the SSBP/1 agent. Preliminary results from this experiment indicate that genetic variation influencing susceptibility is present in the Cheviot sheep.

Fifteen rams were each mated with twenty randomly allocated ewes and were then given subcutaneously 10 ml. of a 10 percent suspension of SSBP/1, 23d pass. After weaning time the ewes were also challenged with the same agent. Before scrapie appeared in these ewes a number had died for various reasons but mainly as a result of pyogenic infection of the brain, so leaving 47 lambs without mothers. These lambs could therefore, in respect of scrapie, only be classified in terms of their sire's response to scrapie challenge. The orphans were subsequently given 2 ml. of a 10 percent suspension of SSBP/1/23 subcutaneously, their ages varying between 2 1/2 and 4 months at the time.

Twenty-four months following inoculation of the rams, the incidence of scrapie in these animals was 40 percent, and it so happened that the 47 orphan lambs were almost equally divided in respect of the negative or positive response of their sires to scrapie challenge. The incidence in the surviving dams was 39 percent at 18 months after challenge. Since it seems reasonable to assume that, so far as scrapie susceptibility is concerned, the dead dams were a random sample of the ewe flock, the occurrence of scrapie in the 47 lambs (table 1) is such as to give clear evidence of a high degree of genetic control of the response of Cheviot sheep to SSBP/1/23 challenge.

The data show a significant association between the susceptibility of rams to scrapie challenge and the susceptibility of their progeny

Table 1a--Incidence of Induced Scrapie in Rams and their Progeny

Progeny \ Sire	Affected	Unaffected
Affected	10	3
Unaffected	11	23

[1] Animal Breeding Research Organization; Director, Animal Diseases Research Association, Moredun Institute; Clinical Investigation Department, Animal Diseases Research Association, Moredun Institute; and Electron Microscopy Department, Animal Diseases Research Association, Moredun Institute, Edinburgh, Scotland, respectively.

to similar challenge (P = 0.02 to 0.01 after making allowance for small numbers).

As the data are limited by the small numbers in the experimental group and by the genetic information being incomplete owing to the impossibility of classifying the dams, it is not possible to draw any firm conclusions about the type of genetic control involved. However, the data are in reasonably close agreement with the possibility that a simple recessive gene controls susceptibility assuming a genetic equilibrium for the parental population and a 60 percent frequency for the recessive allele. This would give a 36 percent incidence of induced scrapie in the parents, a 20 percent incidence in progeny of unaffected rams, and a 60 percent incidence in the progeny of affected rams. These expectations are reasonably close to the observed values of 40 percent, 12 percent and 48 percent, respectively. Further data as it is collected should confirm or refute this possibility.

Whether or not similar results would have been obtained if scrapie from a different source had been used or if another breed of sheep had been selected is unknown. Further, it cannot be assumed that the present results indicate the method of control of natural scrapie as it occurs in the field. The establishment of lines of sheep with a common genetic origin but differing widely in susceptibility to experimental scrapie should assist in determining any connection with proneness to natural scrapie.

REFERENCES

GORDON, W. S. (1960). Report of Special meetings on Scrapie. U.S. Dept. Agr. Rpt. ARS 91 - 22.
Stamp, J. T. (1958). Vet. Rec. 70.
Wilson, D. R., Anderson, R. D. and Smith, W. (1950). Jour. Comp. Path 69: 268.

ADAPTATION OF THE SSBP/1 SCRAPIE AGENT FROM SHEEP TO MICE

A. G. Dickinson and W. Smith[1]
(Presented by A. G. Dickinson)

In view of the large number of experiments which have used the SSBP/1 agent originally isolated by Wilson, it seemed most reasonable 4 years ago, when no mouse-adapted agents were known, to concentrate on trying to adapt this agent to mice rather than turning to other sources of scrapie-affected material, although the outcome of previous attempts to do so at Moredun Institute had not been promising. For instance, 200 random-bred Moredun stock mice challenged intracranially with this agent at weaning had been observed for 2 years and no clinical effects or deaths of a suggestive nature had occurred.

A variety of mouse strains were used for the new series of experiments as shown in table 1.

All the mice were injected intracranially with supernatant of 10 percent SSBP/1/21 brain suspension in saline spun at low speed (500 g.) for 10 minutes.

Uninjected controls were present for C57 and SM stocks, and controls injected with autoclaved inoculum were present for the Moredun strain.

Table 1.--Incidence of Scrapie in mice injected with SSBP/1/21 Scrapie Sheep brain material

Mouse strain	Number injected at birth	Number injected at weaning (3 wk.)	Number developing scrapie-like disease up to 2 years after injection
C57BL.....	5	17	0
SM.....	15	12	0
RIII.....	13	22	2
SS_x/Bt.....	38	25	2
LS_x/Bt.....	32	38	0
F/Bt.....	27	27	0
Moredun random bred	32	29	10

All groups have been observed for at least 18 months and in many cases for more than 2 years, and no undue death rate occurred during that time in any group, apart from the few cases described below where there was clinical evidence of scrapie. No lesions characteristic of scrapie--either neuronal vacuolation, status spongiosus, or oedema of white matter--have been found in any C57, SM, LS_x/Bt , or F/Bt mouse. Clinical scrapie and brain lesions attributed to scrapie are confined to the SS_x/Bt, RIII, and Moredun strains.

The earliest of these lesions was 14 months after injection and the latest 23 months, the average being 18 months. The brains have not all been examined as yet, but judging from results to date it is unlikely that many more cases with characteristic brain lesions will be present. The brain lesions have usually been restricted to status spongiosus and oedema of white matter, both severe in some cases, but neuronal vacuolation has been infrequent and usually absent.

Only one of the mice injected at birth has given any evidence of scrapie lesions--this was an animal of the Moredun strain--the rest were ones injected at weaning.

The clinical syndrome in the mice extended over several weeks and involved an excitable phase followed by an inactive phase; there was moderate loss of body weight and condition, but emaciation was only severe in the oldest mice. There was no evidence of pruritis, and locomotor incoordination, though present in the later phases, was not very marked. Persistent priapism was seen in some.

Thus, the general picture of the incidence is that very few mice showed any scrapie-

[1] Animal Breeding Research Organization Edinburgh, Scotland, and Electron Microscopy Department, Animal Diseases Research Association, Moredun Institute, Edinburgh, Scotland, respectively.

like disease and those which did had an extremely long incubation period. This must be contrasted with the experience of Chandler (1961), and Morris and Gajdusek (1963) who found a scrapie-like disease in mice in a relatively shorter time than in our experiments and with the latter authors there was a very high incidence in several mouse strains. Also Zlotnik and Rennie (1962), in developing the ME 7 strain of agent, have, from the start, had a high incidence of cases.

So far, a passageable scrapie agent has been recovered from two affected mice: one (SS_X/Bt) that developed scrapie 14 months after challenge, the other (Moredun strain) that developed scrapie after 20 months incubation. Brain material from the latter has now produced a high incidence of scrapie in RIII and 2M mice after a 13-month incubation period. The strain isolated from the SS_X/Bt mouse has also produced a very high incidence of scrapie in this case, using mice from the 50 percent inbred 5M strain. Half of the 5M mice developed scrapie at 7 1/2 months after injection and the rest after 12 1/2 months. This bimodality probably has a genetic basis for reasons given in a later section. The recovery of passable strains of agent in inbred or semi-inbred hosts has the advantage that comparisons can be made with other agents as Table 2 shows.

It is unfortunate that the results in table 2 are only based on small groups of mice. However, the standard errors are small and there is other evidence that the ME 7 mean incubation periods are reliable. Taken at its face value, there appears to be interaction between the strains of host and agent in terms of the incubation period but this could change with further passages.

Table 2.--Incubation periods (weeks) of two scrapie agents in different strains of mice

Mouse strain	Mouse adapted[1] SSBP/1 agent		ME 7 agent[2] (From Dickinson & Mackay, 1964)	
	No. of mice	Incubation period	No. of mice	Incubation period
RIII.....	1	56	6	21 ± 0.3
2 M.....	12	62 ± 0.8	4	26 ± 0.7
5 M.....	5 +) 3)	30 ± 0.0 and 52 ± 0.7	8	40 ± 0.8

[1] 1st mouse-to-mouse passage.
[2] 3rd mouse-to-mouse passage.

REFERENCES

Chandler, R. L. (1961) Lancet; i 7191.
Dickinson, A. G. and Mackay, J. M. K. (1964) Heredity; [in press.]
Morris, J. A. and Gajdusek, D. C. (1963) Nature 197: 1084.
Zlotnik, I. and Rennie, J. C. (1962) Jour. Comp. Path. 72: 360.

GENETICAL CONTROL OF THE INCUBATION PERIOD IN MICE OF THE NEUROLOGICAL DISEASE, SCRAPIE

A. G. Dickinson and J. M. K. Mackay[1]
(Presented by A. G. Dickinson)

Numerous attempts have been made during the past 15 years to adapt the scrapie agent to mice: the first to succeed was Chandler (1961) who produced an encephalopathy in mice by inoculating with brain material from affected goats. Scrapie-like diseases have now also been transmitted to mice from a variety of scrapie sources, including directly from affected sheep (Zlotnik and Rennie, 1962, 1963; Morris and Gajdusek, 1963; Dickinson and Mackay, unpublished).

The experiments described in this paper set out to assess the extent to which genetical variation might affect the response of mice to one strain of scrapie agent. Adaptation of scrapie to mice has presented the opportunity for critical bioassay that was not feasible with large animals. The optimum type of animal for this purpose is one with minimum response time, minimum genetic variability, and maximum phenotypic stability to extraneous environmental variables. To this end the extent of genetical determination and the influence of the degree of inbreeding are considered in terms of a quantitative trait appropriate for bioassay purposes.

DETAILS OF SCRAPIE AGENT: ORIGIN, SYMPTOMS, AND NEUROPATHOLOGY

The agent, supplied by Dr. I. Zlotnik, was from the M.E. 7 strain (Zlotnik and Rennie, 1963). This strain originated from brain and spleen of a natural scrapie Suffolk sheep, being forced-fed to mice of a random-bred closed colony at Moredun Institute. The experiment is based on a pool of brains of affected Moredun-stock mice from the second passage by inoculation in these random-bred mice. The incidence of scrapie has been 100 percent at all passages.

The reason for preferring this particular strain of agent are twofold. Neuronal vacuolation is a prominent feature of the disease produced by this strain; the clinical syndrome is very clear-cut, and in this respect more satisfactory than for other strains we have handled.

EXPERIMENTAL DESIGN AND MOUSE STOCKS

Mice were weaned at weekly intervals when 3 to 4 weeks old and inoculated intracranially 2 days after weaning: there were 8 weaning groups. The intracranial dose was approximately 0.02 ml. of the supernatant of a 10 percent suspension allowed to sediment naturally for 1/2 hr. to 2 hr. Of the six mice in any one cage there was usually only one and never more than two of each strain or cross. In the last 4 weaning groups uninjected controls were present and either one or two of the 6 mice could be a control, but they bore no mark by which they could be distinguished immediately without reference to records.

Five highly inbred stocks of mice and four partially inbred strains, with a common random-bred origin have been used. The five standard inbred strains chosen for their diversity were SM/JaxBt, BSVS/Sr, RIII/FaBt, C57BL/FaBt, and LG/JaxBt. In addition, four inbred lines from the Moredun Institute closed colony have been sib-mated for three generations: these are designated 2M, 3M, 4M, and

[1]Animal Breeding Research Organization, and Microbiology Department, Animal Diseases Research Association, Moredun Institute, Edinburgh, Scotland, respectively.

5M. The experiment was designed as a diallel cross--all possible crosses and pure lines--and reciprocal crosses were included wherever the stock numbers permitted. The experiment was designed originally to use the four M lines as a single semi-inbred group in crosses with other strains, but the analysis has shown the need to treat them separately. Therefore the numbers of animals in mating blocks having M lines as one or both parents is unfortunately variable and several of the possible combinations are missing.

CLINICAL CRITERIA

All weaned mice were scored for clinical symptoms of scrapie weekly, during the afternoon, whilst they were being weighed. The scoring system depended entirely on observation of the daytime-inactive phase, and each mouse was scored as either "unaffected," "possibly affected," or "affected." Animals were killed after the third consecutive scores of "affected." Records were designed to prevent knowledge by the operator of the animals' previous scores and mice were always handled in random order.

A number of animals died before the third consecutive "affected" score was reached. In these cases the actual age at death was used in the analysis. It should not be concluded from these results that the clinical diagnosis was unreliable for animals which did not survive till the third "affected" score because very sick animals were kept under daily surveillance.

Forty-six brains of affected animals were chosen at random for pathological examination of the brain as a check on the clinical scorings: all were confirmed positive and we are indebted to Dr. Zlotnik for this assistance.

Only two experimental animals (neither likely to be genetic variants) have failed to develop scrapie out of 718 injected: it may be that these affectively received no inoculum.

RESULTS

The analysis is confined to data on the incubation periods, the term being used here to denote the interval from inoculation to killing as defined above. It is recognised that the incubation period here is confounded with age, since all animals were inoculated at the same age.

Reciprocal crosses were included for formal completeness of the design and not because they were expected to reveal any source of variation. In all, there are 15 pairs of reciprocals, treating the sexes separately, of which none show significant differences. Consequently, reciprocals have been pooled.

Also there is no evidence for an overall difference between the sexes, which is somewhat surprising, but we have some limited observations that lead us to suspect that a sex difference may have emerged if age at death had been used rather than an arbitrary killing criterion: males appear to lose condition very rapidly in the terminal phases in comparison with the females, as shown by observation of their weights.

GENETICAL ANALYSIS

A summary of the results is given in Figure 1. Sex, inoculation groups and reciprocal crosses are pooled for each strain or cross.

There is one missing block in the main 7 x 7 series of crosses, namely SM x LG: since the

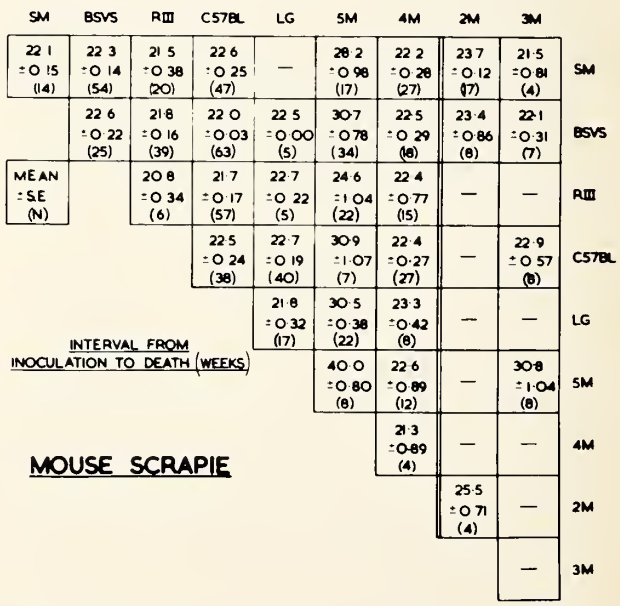


Figure 1.--Interval in weeks from inoculation to death.

two parental strains have almost identical mean values, an intermediate value of 21.9 weeks has been used in the analysis to simplify presentation. Unless there is an unpredictable genetic interaction in this particular cross, this interpolation will lead to slight underestimation of the average degree of dominance of the SM strain.

In view of the deliberate choice of standard inbred strains differing widely from one another, it is surprising that they display such close similarities in response to scrapie. RIII seems to succumb slightly quicker than the four others. In contrast, the 2M, 4M and 5M strains, despite small numbers per group, show significant and marked differences from each other. Almost two-thirds of the strains or crosses involving the four M lines have higher variances than the most variable of the ones involving the other five inbred lines. Since the four M lines were only 50 percent inbred compared with the long history of sib mating in the five standard strains, it is presumed that the variability with the M's is evidence of residual heterozygosity.

It would be reasonable to suspect that the difference in incubation period of the M lines, particularly 5M, from the rest might diminish with further inbreeding as an outcome of inbreeding depression. This seems to be unlikely, as there is no evidence of such depression having occurred in the five standard inbreds judging from the performance of their F's. In addition, there is no evidence that these F's have lower intragroup variances than their parents.

The genetical analysis employed is that derived by Dickinson and Jinks (1956) and applicable to the interpretation of diallel crosses where some parents are not fully inbred. The comparison is used of the variance, V_r, of an array (all the mating types that have one parental strain in common) with the covariance W_r, between that array and the parental strains. The relationship of V_r and W_r is shown in figure 2: all points must lie inside the parabola shown. The effect of a strain being partially heterozygous is that its average degree of dominance is underestimated.

Some of the criteria used in interpretation are indicated on figure 2. On average, C57 and BSVS display no dominance and the same

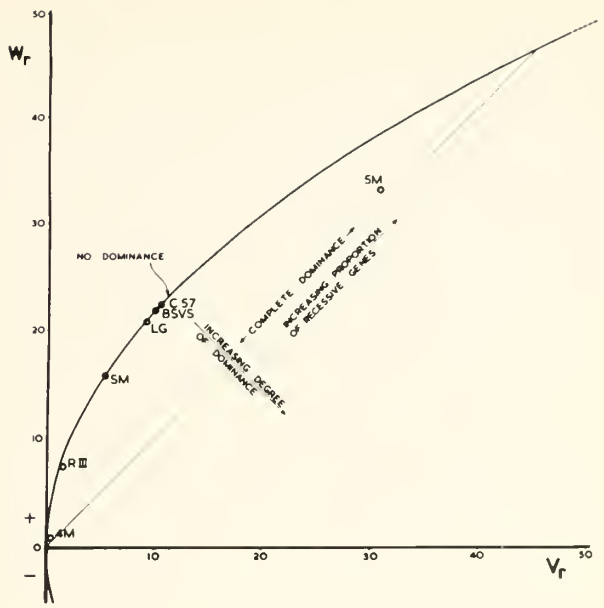


Figure 2.--Relationship between array variance, V_r, and covariance of array with non-recurrent parents, W_r, for each mouse strain.

is almost true for LG. SM shows a small degree of dominance having practically all the slightly dominant genes; much the same applies to RIII but at a much higher degree of partial dominance. Likewise, in view of its position almost on the parabola and near the origin, 4M appears to have virtually all the dominant genes but in this case there is at least full dominance. Finally, for 5M the genetic constitution is very different from the rest: there is a preponderance of recessive genes and the gene action in this strain shows an almost full degree of dominance, which is likely to be underestimated because inbreeding is incomplete.

As judged by the intragroup variances of the inbred lines, the variances of the 4M strain and its crosses are relatively low for a strain that is only partially inbred and this is as expected from the above interpretation of a very high proportion of fully dominant genes in 4M: In contrast the intragroup variances of the 5M strain and its crosses are all high, which again accords with the interpretation given above: namely, that there is considerable residual heterozygosity, in which fully dominant alleles are in the minority.

DISCUSSION

The particular strain of scrapie we have used appears to have high potential for bioassy purposes in any one of these standard inbred strains, and there seems to be no intrinsic value in resorting to the use of F_1 crosses. The type of bioassy applications envisaged relate to understanding the effect of physical and chemical treatment of this remarkably resistant disease agent. By scoring the clinical disease twice weekly and using groups of 20 mice, it should be easy to distinguish 5 percent differences in incubation period. It remains to be shown that change in concentration or structure of the agent used here can be detected as a change in incubation period, but there is some evidence from other work to encourage this expectation (Pattison and Smith, 1963).

Although this work has been carried out using clinical criteria alone, we have no illusions about the difficulties of using solely clinical criteria in most scrapie studies and we fully expect that the considerable effort involved in adopting pathological scoring would have added much to the delicacy of our tests--the duration of the test could probably have been reduced by a quarter.

It appears likely that there is scope for further selection within the 5M line, and a rough estimate based on the diallel analysis and the phenotypic variance is that the incubation period could be increased to at least 45 weeks without any further mutation, simply by accumulating a full complement of recessives and leaving untapped any heterozygosity for genes that show no dominance. The variation between lines could therefore be increased even more than the present twofold difference. The magnitude of the differences between lines can be brought into perspective by considering them as proportions of an average mouse's life span: the most rapid incubation period is about a quarter of a lifetime, the slowest about half of a lifetime. Though difficult to attain in practice, it is easy to visualise selection for increased incubation period reaching a point where the animals die naturally before developing scrapie.

It is interesting to consider some implications of these findings for current research on scrapie, particularly in sheep. At present, we find it necessary to keep experimentally challenged sheep for about 2 years after inoculation to discriminate susceptible individuals from resistant ones. In view of the evidence presented here, this policy may need to be reviewed to avoid classifying sheep as "unaffected," which would become "affected" if a longer interval had been allowed for. The same applies to work on natural scrapie in sheep, but the tactical implications are even more sobering as the incubation period is much longer. Put in general terms, the evidence here calls in question any easy application of the concept of absolute "resistance" in its commonly accepted meaning of failure to develop a disease, where a chronic illness of long incubation period is concerned.

REFERENCES

Chandler, R. L. 1961. Lancet 1: 1378-1379.
Dickinson, A. G. and Jinks, J. L. 1956. Genetics 41: 65-78.
Morris, J. A. and Gajdusek, D. C. 1963. Nature 197: 1084-1086.
Pattison, I. H. and Smith, K. 1963. Res. Vet. Sci. 4: 269-275.
Stamp, J. T. 1962. Vet. Rec. 74: 357-362.
Zlotnik, I. 1962. Acta Neuropath. Suppl. 1: 61-70.
Zlotnik, I. and Rennie, J. C. 1962. Jour. Comp. Path. 72: 360-365.
Zlotnik, I. and Rennie, J. C. 1963. Jour. Comp. Path. 73: 150-162.

A fuller account of this experiment is published in Heredity.

DISCUSSIONS
(Papers Number 30, 31, 32)

Dr. Morris:

What is the initial sign that marks the beginning of the period? What is the sign that the mouse has scrapie?

Dr. Dickinson:

By careful observation of one or two individuals, in fact by running genetically equivalent groups, challenged and unchallenged ones, through overnight activity counters for maximum activity, between 10 o'clock at night and 4 o'clock in the morning, we know that the scrapie mice, the ones which during the day one observes as just sitting there and which don't want to budge--

Dr. Morris:

When does this begin?

Dr. Dickinson:

Those mice which during the day are absolutely immobile, or absolutely immobile when you open the cage to observe them, are more active during the middle of the night than are their controls.

I emphasize this because the first sign you see in the mice with this agent, if you are carefully observing individuals for a prolonged period, is that you get an increase in activity during the daytime. But this is a sign which is extremely easy to miss. It is a transient phase with this agent. It is one which under the conditions which we used for observing and scoring mice you would not see, because we only observed them during the time it took to weigh them. We weighed them during 2 hours on the afternoon of each Tuesday or Wednesday.

So a mouse was only observed from the time the box was open, it was taken out of the box, put on the weigh scale and weighed, the weight recorded, the score recorded.

Activity doesn't come into this.

Increased activity very probably is the earliest sign. This does not enter into our clinical score.

Dr. Morris:

Yes, or do they ever recover and at some later date show the initial signs you just mentioned?

Dr. Dickinson:

Could I leave that to the next paper where we have contact mice? You will see the scoring; how this occasionally happens. The scoring is based on inactivity during the day, though we know it is preceeded by a transient hard-to-score activity phase.

Dr. Dickinson:

Do you mean taking the 5M strain of mice, which take 40 weeks, if we use that brain as the pass agent? I am going on to do this, I have bottles and bottles of frozen brain. This is an important question.

Can I bring the moral out of this which I would like to do, which I have forgotten to do. The moral of this is that if, as I was tempted to do, I had stopped this experiment after half a year, when almost all my mice had gone down, I would have ended up with the interpretation that the 5M line was resistant and that the 2M, 3M, and 4M mice were all susceptible. You can appreciate the point here. Incubation period is a word. And susceptibility and resistance are words. And it may well be that resistance is just incubation period in a susceptible mouse at infinite time.

So this is why I have not been wanting to be tied to the words, "resistance," "susceptibility," and the rest.

You can see the point so far as anybody using random-bred mice is concerned. If you are bioassaying in mice, when you do not know precisely what their genetic control of the incubation period or the agent is, then you are on a risky ground. And if in fact you have the genetic mixture which are present in this ramdom-bred colony, you are liable to come out with weird titration results. So all I can do is recommend using inbreds.

The further recommendation is that there is no evidence whatsoever in my data to show that F_1 mice are any better, have any advantages at all in terms of use for bioassay purposes. Their internal variances are identical with those for the purebred strains.

So it looks like there is no inbreeding depression apropos this characteristic.

Dr. Boucher:

I would like to mention that there has been throughout this meeting some suggestion of parallel. I don't think it is very complete, but there is some suggestion of parallel between the scrapie phenomena and LCM phenomena, or some of them that I have been familiar with. What came to mind was the goat susceptibility and the sheep. We have done some tests with our mice to see how much there might be contamination of controls in the mouse rooms from infected animals. So we very frequently and for long periods left normal animals in various degrees of proximity to the infected ones.

With very few exceptions, in special cases, there has been no crossinfection. In spite of that, if we put guinea pigs in that room, even a long way from the mice, they practically invariably come down in about 3 or 4-- I was going to say days, but they take longer than that.

If we leave them there 3 or 4 days they usually come down.

We have had two cases, one just getting better, of human cross infection from the animals. This is in about 8 years; not a lot. I wonder, I want at some point to raise the question whether there is in anybody's experience the suggestion that scrapie causes any kinds of disease in man.

The LCM mice also show maternal transmission to the offspring, which is the situation which was first noticed when it was a contaminated mouse colony. This has been shown very strongly to be transmission in the female to the fetus.

Another point I want to raise is your question about the genetics of any other examples. I have two examples that I think you will probably throw out as being not quite what you meant. The first, interestingly, is the LCM. We have two strains of mice which are not in any way crossbred, and their susceptibility to somewhat odd late onset of disease due to LCM takes 10 months to show, varies by a factor of 2, in rather similar mice.

The other example I thought of, that you may not be aware of, is the mice of Helier and Lowey in New Zealand. There, as I recollect it, their congenital survey believes this may be a virus. And we have been corresponding. And they admit it may be a virus. Their congenital autoimmune disease produces in about the same time our mice take, a hemolytic anemia, lesions, and various other signs of immunity. They get a very much higher incidence of some of their signs than I think the gene severity, in the F_1 cross with the strains they are using. That would seem to be a possible example.

Dr. Dickinson:

This is good if they are in fact admitting this virus possibility.

Your previous example, your factor of 2, was it timewise? Was it a factor of 2 in incidence or time?

Dr. Boucher:

It is both, actually, if you go into it. The one strain that has the lower incidence does take longer to show the disease.

I think the difference in time is much smaller than the overall difference in incidence. If it is in time, this is not bad, because it is interfered with by a sex difference as well.

The more susceptible strain shows the disease first at 7 months. The less susceptible, as far as I remember, followed about 2 months later. If you measured at 1 year, it has a twofold incidence. The more susceptible has twice as many.

TRANSMISSION BY CONTACT OF SCRAPIE IN MICE

A. G. Dickinson, J. M. K. Mackay, and I. Zlotnik[1]

It is a matter of considerable economic importance whether there is contact transmission between sheep of the neurological disease known as scrapie since scrapie mainly occurs in sheep. However, it has been impossible to demonstrate unequivocally sheep-to-sheep contagion for two reasons; one is the lack of a group of sheep which would be susceptible to scrapie but which are known to be free from the disease; the other is the difficulty of providing a group of sheep with strict isolation from the scrapie agent, due to its considerable resistance to chemical and physical destruction.

It would appear that contact transmission of scrapie from sheep to goats occurs if the goats have lifelong contact with affected sheep from a day old (Stamp, 1962). In this instance at least $3\frac{1}{2}$ years of continued contact took place before definite scrapie appeared in the goats. The interpretation of this result as contagion depends on the apparent great rarity of scrapie in goats that have not been experimentally inoculated with the disease (Mackay and Smith, 1961) and on the fact that scrapie developed in all three breeds of goat reared with the affected sheep. However, since scrapie is such an insidious disease, varying widely in its clinical manifestations and necessitating neuropathological confirmation, some measure of doubt must remain about the presumed absence of scrapie in the general goat population. This difficulty does not arise with mice of standard inbred strains since their breeding histories are known in detail and a scrapie-like disease with the pathology described below is hitherto unknown in uninjected mice. The evidence presented below shows that contact transmission from mouse to mouse does occur.

In a large experiment designed to investigate the genetical control of incubation period of scrapie in mice, various strains and crosses were used and the animals were kept six to a cage, with the sexes separated. In some cages either one or two of the mice present were uninjected controls, but they could not be immediately distinguished as controls. This was important in preventing subjective bias by the observer, since each animal was scored weekly for clinical signs of scrapie. Full details of the experimental design and scoring system are given by Dickinson and Mackay (1964).

The development of scrapie in two of the uninoculated mice--one, a female in cage 93, the other, a male in cage 70--is now reported. In each of these cages five of the mice had been injected intracranially a few days after being weaned and were ear punched with an individual number at 3 weeks - as also were the uninjected controls. The scrapie agent used was ME7, which was originally isolated by forced-feeding of mice with brain and spleen from a naturally affected Suffolk sheep, the agent thereafter being passaged once by intracranial injection of mice (Zlotnik and Rennie; 1962, 1963). Given in this manner the ME7 agent produces 100 percent incidence of scrapie in all mouse types so far tested.

Figure 1 shows the responses of the mice in the two cages under consideration and the life history of each mouse is given in terms of its weekly clinical score for scrapie. The uninjected male (No. 4) in cage 70 was attacked and bitten over the sacral area by its cage mates when it was about 4 months old

[1] Animal Breeding Research Organization, Microbiology Department, Animal Diseases Research Association, Moredun Institute, and Pathology Department, Animal Diseases Research Association, Moredun Institute, Edinburgh, Scotland, respectively.

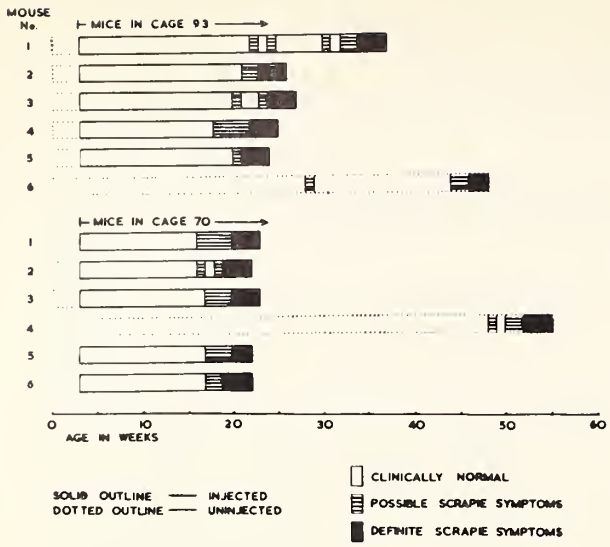


Figure 1.--Life-histories of mice in two cages showing the development of clinical scrapie in both injected and uninjected animals.

and hair growth over this area was sparse for the rest of its life. This is mentioned as a possible route of infection with scrapie. However, fighting is rare amongst female mice, and the uninjected female (No. 6) in cage 93 was not bitten by its affected cage mates.

The pathological changes in the brain were very similar in the two uninjected mice and were confined to certain brain regions. They consisted of neuronal degeneration, including typical scrapie vacuolation, moderate status spongiosus of the gray matter, and limited oedema of the white matter of the cerebellum and of the internal capsule. Vacuolated neurones were present in the lateral vestibular nucleus of the pons and in the pyramidal cells of the hippocampus and cortex, especially in the frontal and hippocampal gyri. Status spongiosus affected many areas, but the most severe lesions were noted in the hippocampus, while moderate changes were seen in the mesencephalon, lateral thalamic nuclei, preoptic region, paraterminal body and in the frontal, hippocampal, and medical olfactory gyri of the cortex.

The above brain lesions, although not very severe, were however, well developed and were identical with those present in mice inoculated with mouse-passaged Suffolk sheep scrapie strain (ME. 7). This similarity was especially striking when the lesions were compared with those seen in mice inoculated with scrapie by the subcutaneous or intraperitoneal routes and destroyed within 1 to 2 weeks after the onset of symptoms.

Since the appearance of clinical scrapie in noninoculated control mice is of fundamental importance in determining the pathogenesis of the disease, it is essential to give careful attention to the possibility of the control animals having been injected by accident.

The possibility of confusion of identity or unintentional injection with scrapie is very improbable. Each mouse could be positively, identified by one or more independent characteristics by reference to records: these were its ear punching and usually also by its coat colour and body weight (strains ranging from 20 to 50g. adult body weight were present in this experiment) so that confusion of identity is unlikely to have occurred.

Consideration of the incubation periods also makes it extremely unlikely that an accidental injection could have been given. It has been established that the incubation period of the ME7 agent, given intracranially to 3 weeks old mice, is under a high degree of genetic control by the host (Dickinson and Mackay, 1964) and this is reflected in the very small standard errors of mean incubation period which are given in table 1 for each of the genotypes under immediate consideration. From figure 1 it will be seen that one of the injected mice (93, No. 1) differed markedly from its injected cage mates in incubation period; the reason for this is genetical as reference to table 1 shows, and its relatively prolonged incubation period has an entirely different origin from that for either of the uninjected mice.

When the individual mice in cages 70 and 93 are considered in terms of deviation of incubation period from the average for injected mice of the same breeding, there is a wide discrepancy only in the case of the two control animals (table 2). Since the statistical probability of this being a chance occurrence is extremely small ($P < 0.001$), it is virtually certain that the control animals received the scrapie agent in a different manner from that of the injected ones.

Table 1.--Mean incubation period for mice injected intracranially at 3.5 weeks old (Dickinson and Mackay, 1964)

Genotype	Incubation period± SE (weeks)
C57BL x 57BL	22.5 ± 0.2
C57BL x RIII	21.7 ± 0.2
C57BL x 3M	22.9 ± 0.6
C57BL x LG...	22.7 ± 0.2
RIII x SM....	21.5 ± 0.4
RIII x 5M....	24.6 ± 1.0
RIII x BSVS..	21.8 ± 0.2
SM x BSVS....	22.3 ± 0.1
5M x 3M.....	30.8 ± 1.0
2M x 2M.....	25.5 ± 0.7
LG x LG.....	21.8 ± 0.3

Since scrapie can be produced by oral transmission of the agent to sheep and goats (Pattison and Millson, 1961) and to mice (Zlotnik and Rennie, 1962), it is important to be able to rule out cannibalism as the cause of the disease in an uninjected animal. This possibility does not arise in the case of the control mice with which this paper is concerned.

ACKNOWLEDGEMENT

We are indebted to Miss Veronica Hamilton-Meikle for her skill in administration of the experimental stock and without whom this work would not have been carried out.

This work is carried out with a grant from the United States Department of Agriculture administered under PL 480.

Table 2.--Comparison of incubation periods for individuals in cages 70 and 93 with the average incubation period for injected mice of similar genotype

Cage and mouse No.	Genotype	Individual incubation period	Status	Deviation from mean incubation period for injected mice of the same genotype
		Weeks		Percent
93/1.....	5M x 3M	[1] 35	Injected	14
93/2.....	RIII x 5M	[1] 24	do	2
93/3.....	RIII x SM	[2] 24	do	12
93/4.....	RIII x BSVS	[1] 22	do	1
93/5.....	C57BL x LG	[1] 21	do	7
93/6.....	C57BL x C57BL	[3] 46	Uninjected	104
70/1.....	C57BL x RIII	[1] 21	Injected	3
70/2.....	SM x BSVS	[1] 20	do	10
70/3.....	C57BL x 3M	[1] 21	do	8
70/4.....	2M x 2M	[3] 53	Uninjected	108
70/5.....	LG x LG	[2] 20	Injected	8
70/6.....	C57BL x LG	[3] 20	do	12

[1] Scrapie diagnosis confirmed by neuropathological examination - advanced lesions.
[2] Mouse found dead: no neuropathological examination possible.
*Scrapie diagnosis confirmed by neuropathological examination - early lesions.

REFERENCES

Dickinson, A. G., and Mackay, J.M.K. (1964). Heredity 19:279.
Mackay, J. M. K., and Smith, W. (1961). Vet. Rec. 73: 394.
Pattison, I., and Millson, G. C. (1961). Jour Comp. Path. 71: 171.
Stamp, J. T. (1962). Vet. Rec. 74: 357.
Zlotnik, I. & Rennie, J. C. (1962). Jour Comp. Path. 72: 360.
Zlotnik, I. & Rennie, J. C. (1963). Jour Comp. Path. 73: 150.

DISCUSSION

Dr. Mayall

I have a slight confusion in these figures of Dr. Zlotnik. Was he feeding sheep material?

Dr. Dickinson:

Dr. Stamp will tell you how these various agents originated.

Dr. Mayall

The other point which should be raised, slightly more general for discussion, is that you have used the terms "resistance" and "susceptibility" as far as your mice, in a somewhat loose manner. I think it possibly should be explained that when one is dealing with transmissible pathogenic agents in a biological system, these are relative terms. You can say that an animal is resistant in comparison to another animal. By this sort of definition your 5M mice could be called resistant mice.

Dr. Dickinson:

I was dealing more with the simplest concept. Resistance meaning absolute resistance. I was trying to query this as you are doing now. I couldn't agree more that you have to talk of relative resistance and degrees of it, and say what you mean.

Dr. Mayall

I would like to pursue this slightly further with regard to these long incubation-period mice. The challenge dose you used was many times the minimum dose required to kill 100 percent of your mice. You could have diluted this out several times.

As you dilute the material out--and I think Dr. Hadlow has published data on this--you get an increased length of the incubation period. I suggest one could arrive at a dilution in which you could still get all your more susceptible mice with the shorter incubation period dying, albeit at a greater age, but that your other ones, your resistant ones, wouldn't die, and this stage in absolute terms you could call them resistant.

Dr. Dickinson:

I couldn't agree more. We have no data on this at the moment. We are just embarking on this, using the same criteria to see whether this in fact works. All the indications are from Compton and Dr. Hadlow, that this in fact will prove to be the case in these situations.

The data that I showed there indicate that at this full dose level you can distinguish groups of about 20 mice after a 20-week period with an accuracy of plus or minus half a week. This, therefore, should enable you to get extreme accuracy in detecting any change which you are able to induce in the agent by dilution or chemical procedures, et cetera, even if it isn't of a large level. You can shift it by as little as 2 weeks and then talk with confidence about it.

Dr. Mayall:

There is a corollary from this as well, and that is one may have an animal that in the field situation is 100 percent resistant to disease and yet by artificial means this animal's resistance can be overcome by subjecting it to a challenge of a massive dose of an agent to which in the normal course of events it would not be exposed.

Dr. Stamp:

One point impinges on this, and one which has given me considerable interest, and that is the fact that both at Moredun and also at Compton we have taken sheep, say Cheviot sheep, where one gets a 30 to 35 percent take, and given the so-called resistant sheep tremendous doses of scrapie inoculum, and I mean enormous doses, but they still don't go down with scrapie.

Our usual injection of scrapie brain seems to be an optimal dose for some reason or other.

Dr. Gordon:

If an animal is resistant when first challenged, you cannot break this resistance with repeated massive doses.

Paper No. 34

THE TRANSMISSION OF SCRAPIE TO MICE AND HAMSTERS

I. Zlotnik[1]
(Presented by J. T. Stamp)[1]

INTRODUCTION

Attempts to transmit scrapie to laboratory animals were unsuccessful for many years, although Wilson (1954) showed that the agent could survive in guinea pigs without, however, giving rise to clinical disease. The first clinical manifestation of scrapie in laboratory animals was reported from Compton by Chandler (1961, 1962), who succeeded in transmitting scrapie from goats affected with the drowsy strain of scrapie to mice. Further work at Moredun (Zlotnik and Rennie, 1962, 1963) was successful in that scrapie was transmitted to mice from goats affected with the scratching syndrome of scrapie, from Suffolk sheep previously inoculated with brain from a natural case of scrapie in a Suffolk sheep, and from a Southdown ewe affected with the natural disease. More recently scrapie has been transmitted from mice to hamsters (Zlotnik, 1963) and from mice to rats (Chandler and Fisher, 1963). Preliminary reports of the transmission of scrapie from a Cheviot ram to mice have been made (Morris and Gajdusek, 1963).

The present paper reviews the experimental work with mice and hamsters which is being carried out at Moredun Institute. All the mice used in the experiment were from a random-bred closed colony of white mice.

TRANSMISSION OF SCRAPIE FROM SHEEP TO MICE

Transmission of Scrapie from Suffolk Sheep Using the Intragastric Route of Inoculation

Two groups of mice, consisting of 16 and 20 animals, respectively, were used. The first

[1]Pathology Department, and Director, respectively, Animal Diseases Research Association, Moredun Institute, Edinburgh, Scotland.

group received 30 percent spleen suspensions obtained from scrapie-affected Suffolk sheep, the other group 30 percent brain suspensions obtained from the same sheep. The materials were given directly into the stomach, using a syringe with a large-bore needle. The spleens and brains were subsequently fed to the mice twice weekly for 11 weeks. None of these animals developed clinical symptoms in the following 13 months, and at this time some of the mice in each group were destroyed and their brains examined histologically. Neuronal degenerative changes and/or status spongiosus were found in all brains. The remaining mice were allowed to live for a further 2 months, but since no definite clinical symptoms developed it was concluded that the animals had a protracted subclinical form of scrapie.

Brain and spleen and mixed brain and spleen pools were made from these mice, the pools being again inoculated into the stomach of further mice, while, in addition, 10 percent suspensions were given to groups of weaned mice, using either the intracerebral or intraperitoneal routes (ME 7, 8, 9, 10, 11, and 12). Between 5 and 6 1/2 months following the intracranial inoculations and between 9 and 9 1/2 months following the intraperitoneal injections, symptoms characteristic of a neurological disease appeared which caused death of all the mice. The length of the clinical illness varied from a few days to several weeks. Three definite syndromes were seen - the hyperexcitable type, the fat type, and the lethargic type. The hyperexcitable form of the disease ran a very rapid course, the fat form ran a chronic, protracted course, and the lethargic form, which was the commonest, usually lasted a few weeks.

Further subinoculations of brain material from these second-passage mice were made (ME 7, 8, and 10), but one, ME 7, was chosen as the most suitable strain for further work. Up to the present, six passages in mice of ME 7 have been made, and the incubation period has varied from 4 to 5 months (fig 1).

Supernate, boiled for 30 minutes, of the brain suspensions made from the first-passage mice (subclinical cases) was also inoculated intracranially into 60 mice. Clinical signs of scrapie followed in 9 months. The disease was very protracted, and the mice remained alive for 6 weeks before the first death occurred. The brains of these mice removed 10 1/2 months after inoculation showed very widespread lesions of typical neuronal vacuolation in many subcortical centres. On the other hand, the boiled supernate of brain suspensions taken from mice of the third passage (ME 7) produced a very severe clinical disease in 7 months and death followed quickly. The reasons for these differences are as yet unknown.

Transmission of Scrapie from Suffolk Sheep Using the Intracerebral Route of Inoculation

Three such transmissions have been attempted as follows:

(1) In the first experiment, newborn mice were given intraperitoneal inoculations of 0.1 ml. of a 10 percent suspension of either brain or spleen homogenates taken from a normal Suffolk sheep, in an attempt to render the mice immunologically tolerant to normal sheep tissues. As soon as the mice were weaned they received intracerebral inoculations of 0.03 ml. of either brain or spleen homogenate from a Suffolk sheep destroyed in an advanced stage of scrapie. Since symptoms were absent in these mice 12 months after scrapie inoculation, they were destroyed and the brains examined histologically. Limited brain lesions were found in all the mice, irrespective of whether they had been inoculated with spleen or with brain of the scrapied Suffolk. Portions of these brains (ME 2) were pooled and were further inoculated into untreated weaned mice. No symptoms having appeared in these mice during 10 months postinoculation, the mice were destroyed and typical brain lesions were found in 70 percent of the mice. These brains have been inoculated into normal unweaned mice and up to the present (7 months) symptoms have not as yet occurred.

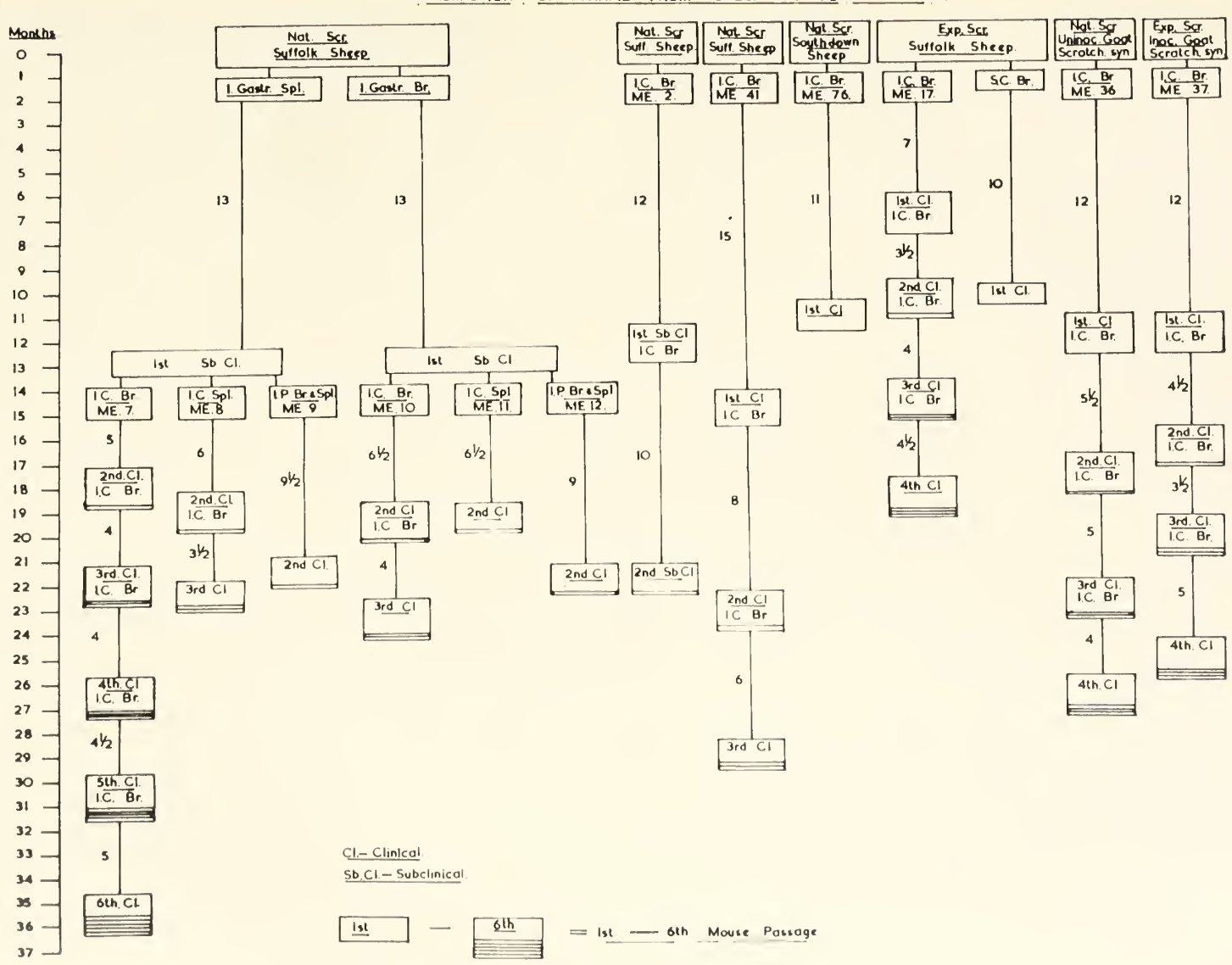

TRANSMISSION OF SCRAPIE FROM SHEEP AND GOATS TO MICE.
Months
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
Nat. Scr. Suffolk Sheep
I. Gastr. Spl.
I. Gastr. Br.
13
13
1st Sb Cl.
1st Sb Cl
I.C. Br. ME. 7.
I.C. Spl. ME. 8
I.P. Br. & Spl ME. 9
I.C. Br. ME. 10
I.C. Spl ME. 11
I.P. Br. & Spl ME 12.
5
6
9½
6½
6½
9
2nd. Cl. I.C. Br.
2nd. Cl. I.C. Br
2nd. Cl.
2nd Cl I.C. Br
2nd Cl
2nd Cl
4
3½
4
3rd. Cl.
3rd Cl
3rd. Cl. I.C. Br
3rd Cl
4
4th. Cl I.C. Br.
4½
5th. Cl. I.C. Br.
5
6th. Cl.
Nat. Scr Suff. Sheep
I.C. Br ME. 2
Nat. Scr Suff. Sheep
I.C. Br ME 41
Nat. Scr. Southdown Sheep
I.C. Br. ME. 76.
Exp. Scr. Suffolk Sheep.
I.C. Br. ME. 17.
S.C. Br.
Nat. Scr Unimac. Goat Scratch. syn
I.C. Br ME 36
Exp. Scr. Inoc. Goat Scratch. syn
I.C. Br. ME 37.
12
15
11
7
10
12
12
1st Sb Cl I.C. Br
1st Cl I.C. Br
1st Cl
1st Cl. I.C. Br.
1st Cl
1st Cl. I.C. Br
1st Cl. I.C. Br
10
8
3½
4
5½
4½
2nd Sb Cl
2nd Cl I.C. Br
2nd. Cl. I.C. Br.
2nd. Cl. I.C. Br
2nd. Cl. I.C. Br
6
4½
3rd Cl
3rd. Cl I.C. Br
3rd. Cl. I.C. Br
3rd Cl. I.C. Br
5
4th Cl
4th. Cl
4th. Cl
5
Cl.– Clinical.
Sb.Cl.– Subclinical.
1st
6th
= 1st —— 6th Mouse Passage

Figure 1.

(2) Intracerebral inoculation of 3-week-old mice with a 10 percent homogenate of a brain taken from a Suffolk ewe destroyed in extremis following a very short period of peracute symptoms of natural scrapie was made. All the inoculated mice remained healthy for almost 15 months, when a few animals began to be unable to move. The mice were destroyed and characteristic lesions of scrapie were found in all the brains. Intracranial inoculation of this brain material into further mice produced the fat syndrome of scrapie in all the mice after a postinoculation period of 8 months. A further passage of brain suspensions produced a further shortening of the incubation period to 6 months, and, although not all mice suffered from the fat syndrome, the disease ran a very chronic and protracted course.

(3) Intracerebral inoculation of two groups of mice with a Suffolk sheep brain suspension, the brain having been taken from a Suffolk ewe affected with a very acute form of scrapie following inoculation with scrapie brain (ME 17), was made. Mice of one group had been inoculated intraperitoneally at birth with a suspension of brain from a normal Suffolk sheep, while mice of the other group were previously untreated. Seven months after the intracranial inoculations, mice of the group that received normal brain at birth began to show symptoms and in the untreated group symptoms appeared 2 weeks later. Histological examination showed brain lesions characteristic of Suffolk-type scrapie. Three further passages in mice have been made, and at each step the incubation period has been between 3 1/2 to 4 1/2 months.

Subcutaneous inoculation of mice with the same scrapie sheep brain homogenate caused scrapie to appear in the mice after an incubation period of 10 months. Similar supernates of brain homogenates in normal saline solution at 4^{o} C. for 14 days were inoculated intracranially. All the mice developed the disease after 7 1/2 months. The supernate obtained following the centrifugation at 200 g. for 15 minutes of boiled (30 min.) scrapie sheep brain homogenate, also centrifuged before boiling, when inoculated intracerebrally into mice produced clinical signs 7 1/2 months later.

Transmission of Scrapie to Mice From Sheep of Breeds Other than Suffolk

Up to the present the only successful transmission of scrapie to mice from breeds of sheep other than the Suffolk has been from the brain of a scrapied Southdown ewe showing acute clinical symptoms. Twenty mice were inoculated intracranially and the mice were clinically affected before 11 months. Histological examination of their brains showed typical lesions of scrapie, which were very widespread and present in all subcortical centres.

A number of other attempts to transmit scrapie to mice from other breeds of sheep have so far been unsuccessful. The brain material used was obtained from natural cases of scrapie in Cheviot, Swaledale, and Half-bred sheep, from experimentally infected Cheviot sheep, from normal Cheviot and Suffolk sheep, from lambs affected with swayback, and from sheep with experimental allergic encephalitis. Many of the mice were sacrificed at various periods between 12 to 14 months and their brains examined histologically but no lesions suggestive of scrapie have been found.

TRANSMISSION OF SCRAPIE FROM GOATS TO MICE

Two transmissions of scrapie from goats to mice have been made, and each has been passaged in mice four times.

In the first transmission, brain material (ME 36) from a spontaneous case of scrapie in an uninoculated goat (Mackay and Smith, 1961) was inoculated intracranially into mice. Apart from some indefinite signs of ill health, no definite symptoms appeared in these mice for 12 months following the inoculation. When they were killed lesions were present in all the mouse brains, and intracerebral subinoculation of this material into weaned mice produced a fatal disease with severe symptoms in 5 1/2 months. In subsequent passages the incubation period was 5 and 4 months, respectively.

In the second transmission brain material from three goats inoculated experimentally with the scratching syndrome of scrapie and

showing advanced symptoms of the disease was inoculated into mice, and a fatal form of the disease followed within 12 months (ME 37). Three further passages through mice resulted in a shortening of the incubation period to 4 1/2 months, 3 1/2 months, and 5 months respectively.

The clinical symptoms and the brain pathology were similar to those of mice inoculated with Suffolk sheep strains.

TRANSMISSION OF MOUSE-PASSAGED SCRAPIE TO NEWBORN MICE

Fifteen mice 2 to 6 hours old were inoculated intraperitoneally with second-mouse-passaged type scrapie (ME 37). Scrapie developed in 10 of these mice 6 1/2 months later. All the mice were destroyed and brain lesions were present, but only in the 10 mice showing symptoms.

Sixteen newborn mice were inoculated intraperitoneally with second-mouse-passaged (ME 7) Suffolk sheep strain. Scrapie appeared 6 months later in some of these mice; all were destroyed, and widespread scrapie lesions were present in 7 brains and absent in 9.

TRANSMISSION OF MOUSE-PASSAGED SCRAPIE TO GOLDEN HAMSTERS

Six hamsters were inoculated intracerebrally with second-mouse-passaged scratching goat strain scrapie (ME 37).

Six hamsters were similarly inoculated with third-mouse-passaged natural Suffolk sheep scrapie (ME 7).

Six hamsters were similarly inoculated with second-mouse-passaged Suffolk sheep scrapie following inoculation (ME 17).

All the inoculated hamsters developed symptoms and either died or were destroyed in extremis. The earliest symptoms occurred 9 months after inoculation in the hamsters inoculated with ME 37; 4 weeks later, symptoms appeared in the hamsters of the other two groups. The hamsters were drowsy and curled up; when roused, they moved with a very unsteady gait and kept the hind legs spread out sideways. Death occurred either very quickly after the fast development of symptoms or was preceded by a short period of complete prostration.

TRANSMISSION OF MOUSE-PASSAGED SCRAPIE TO GOATS AND SHEEP

Mouse-passaged scrapie brain suspensions were inoculated intracerebrally into the following animals:

Four goats were given the natural Suffolk sheep scrapie (ME 7) passaged twice in mice, once by forced feeding and then by intraperitoneal inoculation. In neither pass was inoculum given into the brains of the mice.

Four goats were inoculated with the scratching goat type of scrapie passaged intracerebrally twice in mice (ME 37).

Four goats were inoculated with Suffolk sheep scrapie following inoculation passaged once in mice (ME 17).

All the goats developed scrapie within 8 to 9 months following inoculation. Symptoms were first observed in the goats inoculated with ME 7 and 4 weeks later in the other goats. All the goats suffered from a severe itch which caused them to bite their skin. Various degrees of incoordination were seen. Lesions of scrapie were present when the brains were examined histologically.

Twenty 6-month-old Cheviot sheep were inoculated subcutaneously with the same material (ME 7) as were the goats in the first category. Thirteen months later one animal has developed clinical scrapie.

STUDIES ON THE PROPERTIES OF THE SCRAPIE AGENT IN MICE

Infectivity of Mouse Organs after Intracerebral Inoculations

Six mice in an advanced stage of scrapie following intracerebral inoculations with second-mouse-passage of a natural Suffolk strain of scrapie were destroyed and the brain, spleen, intestine, kidneys, and liver of each removed. Tenfold dilutions (10-1 to 10-7) of suspensions of the tissues were inoculated intracerebrally into groups of nine mice. Mice inoculated with brain were the first (4 1/2 months) to develop symptoms; those inoculated with similar dilutions of gut and spleen did so a month later. At six months following the inoculations all the mice were examined post mortem and the titration results,

based upon the presence or absence of histological brain lesions (MHD), were:

Brain homogenates:	MHD 50 = $10^{-5.8}$
Gut homogenates:	MHD 50 = $10^{-2.9}$
Spleen homogenates:	MHD 50 = $10^{-2.9}$
Liver and Kidney:	No lesions present

Infectivity of Brain and Spleen After Intraperitoneal Inoculations

Three of twelve mice inoculated by the intraperitoneal route with scrapie mouse brain (ME 7) were destroyed at monthly intervals for 4 months, their brains and spleen being each inoculated into further mice. The spleen of mice killed at 1 month and reinoculated caused scrapie to appear at 5 1/2 months, whereas the brains removed at the same time caused no illness in 12 months. The spleen of mice killed at 2 months caused scrapie to appear at 4 1/2 months, while the brains removed at the same time produced the disease in 6 months. The mice inoculated with spleen and brain taken at 3 and 4 months developed scrapie in 4 1/2 months.

Neutralisation Experiments

A comparison of the infectivity of scrapie mouse brain (natural Suffolk scrapie) suspension in normal saline, in serum from a normal Suffolk sheep, and in serum from a sheep affected with natural scrapie was made. Tenfold dilutions (10-1 to 10-7) of each suspension were left at room temperature for 4 hours and were then each inoculated into 9 mice. All mice were destroyed at 5 months and the titration results were as follows:

Scrapie agent in saline:	MHD 50 = $10^{-5.3}$
Scrapie agent in normal sheep serum:	MHD 50 = $10^{-5.6}$
Scrapie agent in natural scrapie Suffolk sheep serum:	MHD 50 = $10^{-5.0}$

A similar experiment using serum from rabbits given multiple intramuscular injections of scrapie sheep spleen in Freud's adjuvant was carried out, the period of neutralisation being 2 hours at room temperature. The results were as follows:

Scrapie agent in saline:	MHD 50 = $10^{-5.15}$
Scrapie agent in normal rabbit serum:	MHD 50 = $10^{-6.3}$
Scrapie agent in rabbit anti-scrapie sheep spleen serum:	MHD 50 = $10^{-6.0}$

REFERENCES

1. Chandler, R. L. (1961) Lancet 1: 1378.
2. Idem. (1962) Lancet 1: 107
3. Chandler, R. L., and Fisher, Jacqueline (1963) Lancet 2: 1165.
4. Mackay, J. M. K., and Smith, W. (1961) Vet. Rec. 73: 344.
5. Morris, J. A., and Gajdusek, D.C. (1963) Nature 197: 1084.
6. Wilson, D. R. (1954) Unpublished work.
7. Zlotnik, I. (1963). Lancet 2: 1072.
8. Zlotnik, I., and Rennie, J. C. (1962) Jour. Comp.Path. 72: 360.
9. Zlotnik, I., and Rennie, J. C. (1963) Jour.Comp.Path. 73: 150.

DISCUSSION

Dr. Rea:

Dr. Stamp, do you observe any difference in the clinical picture when you take this agent through either rat or goat and back into sheep, or into any other animals for that matter? Do you see any observable difference in so far as clinical signs are concerned, rather than incubation time?

Dr. Stamp:

The only time that this agent has been taken through mice into sheep is the one that I have described, and I don't think there was any difference. I think Dr. Dickinson can probably answer the mouse questions better than I can. I wouldn't have thought it would make any difference. I think it is the agent rather than the pass that makes the difference.

Dr. Dickinson:

The second pass on the SSBP/1 in mice differs from the first pass in mice. My first pass in mice resembles to my mind more the kind of pictures that I have seen of Chandler's agent in mice. The second pass of agents in mice on which I have not seen the neuropathology yet, I have little doubt it is scrapie, resembles clinically the ME7 agent. So there are differences that accrue with passage.

Dr. Plummer:

Dr. Stamp, the extent of lesions found in the sheep, I suppose they could be interpreted as an increased virulence of the agent. If it is, it might mean that the mouse tissue would eventually make a good antigen for serological work. I wonder if you have ever examined sheep feces for the presence of virus. This could be a way of seeding down the pasture.

Dr. Stamp:

I think the answer to your first question is that the lesions are much more characteristic of scrapie of Suffolk strain in Cheviots rather than a "hotting up" of the agent. This is the way I would see it at the moment since the lesions are characteristic of the strain of agent used in the mice. I don't think the lesions in the sheep receiving mouse material for instance are any different than the lesions that would have been seen had we put the original Suffolk sheep material into the Cheviot sheep.

Dr. Plummer:

I thought you said there were more vacuoles--

Dr Stamp:

More vacuoles are seen than in Cheviot sheep inoculated with Cheviot material. I am trying to emphasize that Suffolk ME7 into mice, from mice into mice, and from mice into sheep still end up giving the Suffolk pathology, not the Cheviot pathology.

Your second suggestion, I am not sure whether it is in hand. Dr. Gordon, you were doing something with feces.

Dr. Gordon:

We have done some work in the examination of secretions and excretions from affected goats. Feces, urine, milk, and saliva have all been extensively examined by Pattison. The only clue or suggestion that the agent was detectable in any of these materials was a goat that had received feces. We both thought this animal was showing early clinical signs of scrapie for a considerable period, but the condition failed to become progressive and we cannot tell if this was a mild manifestation or not.

On the question of returning mouse brain agent to goats and sheep, the drowsy goat strain which was successfully passed to mice was then returned to three sheep (the progeny of two susceptible parents,) and also several goats. The goats developed scrapie, confirmed histologically. All three sheep developed symptoms of an excephalopathy 22 to 25 months after inoculation. While they had locomotor symptoms of scrapie, only one showed evidence of rubbing. Histologically, vacuolation of nerve cells was not a feature but status spongiosum and nerve cell degeneration reminiscent of the lesion produced by drowsy goat strain material was evident.

Dr. Stamp:

I would agree that the difficulty of transmitting the agent from one species to another is quite great on occasion. There is no doubt about this. Sheep to goats, for instance, and goats back to sheep, is not very easy to achieve. I think it is true to say that my report here of the mouse to sheep pass and what Dr. Gordon has just reported are the first examples that show that mouse scrapie can in fact produce scrapie in sheep.

Mr. Yohe:

The case you were quoting that had been passaged through the mice and then back to the sheep and showed signs of being like the Suffolk strain was this Cheviot strain?

Dr. Stamp:

No, it was the Suffolk strain, the ME 7 Suffolk strain. The Suffolk strain passed through mice and back into sheep has still the characteristics of Suffolk. May I go back to Dr. Plummer's question of a moment ago, we think that Zlotnik's finding that positive results follow the injection of intestines of mice would indicate that feces may in fact transmit scrapie.

AN INVESTIGATION INTO THE NATURE OF THE CAUSAL AGENT OF SHEEP SCRAPIE AND SOME OBSERVATIONS ON THE BIOCHEMISTRY OF THE DISEASE

D. L. Mould and J. S. Slater [1]
(Presented by J. T. Stamp)[2]

An investigation into the precise nature of the causal agent of sheep scrapie, limited by the time delay before the onset of the disease, is still a long term project that can be extended even further by the desire to correlate experimental facts into a logical background. The work to be described is based on that being carried out at the Moredun Institute and summaries a biochemical attitude and approach to both the problem of the extraction and purification of the agent and the associated biochemistry of the development of the disease in the animal. Much of this work has not hitherto been published or is, at present, in the course of publication. The initial experimental facts concerned with the disease in sheep were that (1) it could be transmitted experimentally by intracerebral or subcutaneous inoculation of healthy sheep with preparations of diseased tissue; (2) it could be transmitted and passaged through goats; (3) the process of natural transmission was unknown but there appeared to be involved an inheritable factor conferring susceptibility or resistance to the disease; (4) the variable response of individual sheep, coupled with the long period of development of the disease, made titration of activity virtually impossible; and (5) it was accompanied by a very characteristic degeneration of neuronal tissue.

A biochemical investigation of scrapie has attempted to approach the whole problem from three different directions.

1. The extraction, purification and characterisation of the scrapie agent. Since high activity was consistently located in the sedimented pellet after centrifugation, the purification methods have tended to follow standard virus procedures. Until transmission to the mouse (Chandler, 1961, 1963) and its subsequent use as a biological test of activity, this type of work was limited in scope. An approach to fractionation and purification studies involving an estimate of recovery of infective agent at each stage of the procedure was difficult to achieve and possible experiments were devised with the ultimate object of only being able to test the final preparation or a selected intermediate stage.

2. The clinical biochemistry of scrapie. Apart from the inherent importance of a detailed clinical biochemical study of experimentally infected animals during the development of the disease, the elucidation of a characteristic clinical biochemical change in a scrapie-affected animal could lead to a useful diagnostic aid. There is also the extremely practical question as to the reasons underlying the relation between resistance and susceptibility in individual sheep and the selection of stock possibly on a genetic basis.

3. Biochemical changes in the central nervous system. The slow progressive degeneration of neuronal tissue could be regarded as a metabolic malfunction, wholly or in part related to the incidence of a virus-like agent, and might have ramifications in other types of neuropathological conditions. Metabolic products could conceivably lead to changes in constitutents detectable by clinical biochemical observations.

[1]Physical Chemistry Department, Animal Diseases Research Association, Moredun Institute, Edinburgh, Scotland.
[2]Director, Animal Diseases Research Association, Moredun Institute, Edinburgh, Scotland.

Progress in the Extraction, Purification, and Characterisation of the Scrapie Agent

Earlier experimental procedures were directed toward the purification of the scrapie agent from sheep and goat brain tissue by a butanol extraction method followed by centrifugation similar to that used originally by Bachrach and Schwerdt (1952) for the purification of mouse-adapted Lansing poliomyelitis virus from cotton rat central nervous system. The results from both species were in close agreement and demonstrated that the active agent could be precipitated at pH 4.5, withstood treatment with butanol, and could be partially eluted with 1 M NaCl at pH 7.0. A high proportion of the activity was obtained in the final sedimented pellet (80,000 g. for 2 hr.). A considerable amount of activity remained in the solvent-extracted residue and could be further removed by salt elution. Activity remained in the sediment pellet after repeated ultracentrifugation cycles, and the general conclusion was made that the active agent was associated with a particulate entity either existing independently or in close combination with undefined tissue fragments (Mould and Smith, 1962 a, b).

Similar findings have been confirmed and extended by the recent results of Hunter, Millson, and Meek (1963) that mouse scrapie activity has been found to reside in the mitochondrial size fraction from scrapie mouse homogenates. We have reached the same experimental conclusion, apart from postulating a wider spread of active tissue components, from similar studies using, however, a different strain of mouse scrapie (ME 7) derived from a naturally occurring case of scrapie in Suffolk sheep (Zlotnik and Rennie, 1963). This differs from the strain used by Hunter et al. (see also Eklund, Hadlow, and Kennedy, 1963) in that the histopathology of the disease in mice is indistinguishable from that in the original sheep host. The observation that scrapie activity held in sheep and goat tissue sediments could be partially recovered by extraction with buffered saline led to the possibility of adsorption of the tissue suspensions on calcium phosphate columns, followed by elution with progressively increasing phosphate concentration according to the technique already successfully used as a method of virus purification (Taverne, Marshall, and Fulton, 1958). A convenient medium was thereby provided for studying the effect of elution with increasing salt concentration at controlled pH and also for characterising any virus-like nature of the scrapie agent that might be exhibited by the chromatographic adsorption of the activity on the column. The adsorption behaviour of scrapie activity in sheep, goat, and mouse brain tissue preparations have been investigated in detail by biological testing in sheep and mouse (Mould, Smith, and Dawson, 1963).

In order to preserve activity, no attempt was made to release the agent by chemical action and preparations applied to the columns were resuspended centrifuged pellets of microsomal dimensions. In two experiments ether extraction of tissue homogenates were carried out for the removal of lipids. A loss of activity was assumed, but balanced against this was the possibility of a release of scrapie agent by solvent action. The general conclusion was that elution of centrifuged preparations of scrapie brain tissue from sheep, goats, and mice through calcium phosphate columns did not provide a method for the concentration of the biologically active agent. A purification (10-fold) of the agent was observed in 0.05M phosphate eluate of mouse scrapie tissue. The activity was always found in the pelleted sediment after high speed centrifugation of the eluates. After ether extraction of the tissue, both sheep and mice eluates appeared to behave somewhat similarly when biologically tested in the homologous animal. The elution behaviour was modified after ether treatment. Results tended to suggest that the elution characteristics of the scrapie agent present in sheep, goat, and mouse brain may differ. A buffer extraction procedure applied to scrapie sheep brain gave an inoculum of good potency when tested in sheep (Mould and Smith, 1962 a). The same extraction carried out with scrapie goat brain was apparently quite inactive in goats, while a mouse brain extract was only very weakly active. These observations are in agreement with complete elution of activity from sheep material with 0.05M phosphate, poor elution of activity from goat material with 0.05M phosphate followed by elution of activity at higher concentrations, and elution of activity from mouse material only at 0.4M phosphate.

Considerably more quantitative data is required on various aspects of this work, and having established the properties of intact tissue fragments the effects of disrupting bound scrapie active material by various chemical and physical methods are at present under investigation.

All experimental work on scrapie in mice involving titration of activity is being carried out with a Suffolk strain as mentioned above. Estimation of scrapie incidence is made from histological evidence (Zlotnik and Rennie, 1963), all groups of mice being slaughtered at the same time, either 5 months or 6 months postinoculation. This method eliminates any error due to misinterpretation of clinical symptoms by defining the incidence of the disease in terms of the pathological appearance and reduces the duration of an experiment to about 6 months. Strict comparisons of titre are thus possible at definite fixed times after inoculation. The LD_{50}, defined as that dilution of material which induced scrapie in half the animals inoculated, is lower than estimations made from clinical symptoms or histology after observing the mice for longer periods, as might be expected from the increase in incubation time with dilution.

Techniques employing centrifugation in sucrose density gradients for the fractionation of brain tissue cellular constituents (Hunter, Millson and Meek, 1963; Whittaker, 1959) have also been used for the location of the scrapie agent in mice. Final titrations are only now being analysed, but it appears that the activity is spread over a range of particle dimensions in the mitochondrial-ribosomal groupings rather than in the mitochondrial range itself. The results are quite comparable with those of Hunter et al. (1963) when both are considered in relation to variations in homogenisation technique and also to possible variability in the location of the agent within the tissue as exemplified by the different distribution of histological lesions in the two types of mouse scrapie being investigated. Within the wide limits imposed by the number of mice at each dilution level (7) and the variance in response, recovery of biological activity is good and a ten-fold purification of the agent, expressed in terms of protein, has been obtained. Only differences of 0.6 - 1.0 in LD_{50} should be regarded as significant.

Activity can, however, be lost in preparative procedures during low-speed centrifugation for the removal of large tissue debris. For various experimental reasons, 10 percent (w/v) homogenates were prepared by different procedures from a pool of scrapie mouse brains finely ground together in the frozen state. The supernatant from an homogenate in 0.32M sucrose after centrifugation for 5 min. at 500 g. gave LD_{50} of - 5.03 after 5 months; homogenisation in 0.85 percent saline followed by centrifugation for 30 min. at 500 g. gave LD_{50} of - 3.34 after 5 months; homogenisation in 0.85 percent saline followed by centrifugation for 1 min. at 25,000 r.p.m. gave LD_{50} of - 4.46 after 6 months. This raises the possibility that the scrapie agent may be located within the structural elements of the tissue rather than within the intra-cellular components, bearing in mind that these experiments were carried out with frozen tissue.

A random-bred stock colony of Swiss white mice have been used for the mouse scrapie investigation. There appears to be a larger biological variation in susceptibility to inoculation, noticeable with decrease in scrapie potency or increase in dilution of the inocula, than has been reported by other workers. This could be a property of the different scrapie strain in use, but accumulated evidence has tended to support a variability in the mouse as more probable. Although experimentally this is of no advantage, it is of interst that the behaviour of this strain of scrapie in the mouse is similar in degree to that of the original strain in goats and sheep.

Throughout the whole progress of this work, many preparations and extractions have been examined by electron microscopy. Originally this was done with the object of searching for an identifiable particle in more varied scrapie preparations than could ever in practice be subjected to biological testing. With the development of scrapie in mice it is now possible to examine in more detail preparations known to be biologically active in the mouse. A blind search for an object of unknown morphology involves considerable photography and the observations have no claim to be exhaustive, but so far no positive conclusion has been reached. Sheep, goat, and mouse brain, and occasionally spleen tissue, preparations have been examined, and purification procedures have

involved both column chromatography and density gradient centrifugation in sucrose or CsCl. When quantitative measurement of biological activity and purification was achieved with the mouse, it became obvious that a stage in the purification of the active agent is only now being attained in which unequivocal results from electron microscopy might be possible.

During the study of adsorption behavior on calcium phosphate columns, whole sheep brains were homogenised and extracted with ether. The extracted residue was resuspended in 0.05M phosphate, pH7, and sedimented by high speed centrifugation. The final pellet was resuspended in 0.05M phosphate and applied to a calcium phosphate column. The column was eluted stepwise in increments of 0.1M phosphate concentration from 0.1M to 0.5M. Electron microscopic examination of the eluates consistently showed the presence of regular disc-shaped bodies 100 to 200 mμ in diameter in the 0.1M to 0.3M eluates from scrapie-affected tissue. Normal brain tissue preparations showed no trace of these particulate entities (Mould, Smith, and Dawson, 1963). Negative staining by phosphotungstic acid has not demonstrated any recognisable subunit structure. The particles have been purified further by density gradient centrifugation in CsCl. The 0.2M eluate has been sedimented and the resuspended sediment pellet layered on CsCl (40 percent w/v). After centrifugation the bodies are left in the top layers only of the density gradient.

The morphological appearance of the particles is very similar to those found in liver and classified as lysosomes (Daems and Van Rijssel, 1961; Novikoff, 1961). Studies on enzymic activities likely to be associated with the particles extracted from brain tissue have not been conclusive, as there has always been the possibility in the experimental techniques of the activity arising from other cellular constituents. Very little information is available as to the stability and existence of lysosomes in brain tissue. The function of these particles in scrapie sheep brain and whether their appearance is a manifestation of the degenerative process involved are matters for speculation.

No similar entities have been recovered by similar techniques from scrapie mouse brains. Results of titration experiments in mice to determine whether they are specifically associated with scrapie activity will take time, as allowance has to be made for the loss of activity (10^{-2}) due to ether treatment, together with the probability of a slow response in the mouse. Biological testing in the sheep has already been reported, but in the absence of titration the numerical data can be misleading.

Clinical Biochemistry of Scrapie

There are several reasons for attempting a clinical biochemical study of this disease, and when these are examined it seems surprising that work of this nature has only recently begun to gather momentum.

Clinical chemistry may have a contribution to make to the aetiology of scrapie. This approach has been employed by Avery and Darcel who, in order to select the tissue or body fluid constituents to be analysed, have drawn several analogies between scrapie and other disorders of the central nervous system or, from the genetic viewpoint, have tried to link incidence of scrapie to particular haemoglobin or potassium types in sheep. The recent reported infection of sheep with scrapie after inoculation with a preparation from sclerotic human brain offers new possibilities (Field, Miller, and Russell, 1962; Campbell, Norman, and Sandry, 1963). Biochemistry may strengthen the virus theory of scrapie in a way that would not have been possible 10 years ago. Recent advances in the enzymology of virus-infected tissues (Bradley, 1957; Allison and Sandelin, 1963) show that in certain cases the onset of an infection might be detected by measuring enzymic activities. Progress in the metabolism of cultured cell-virus systems, especially nucleic acid function, has been made, and again the biochemical study of cells "infected" in vitro with scrapie could be of great value, particularly in present circumstances where a cytopathic effect is absent or slow to develop.

There is still need to reduce the time factor involved in animal inoculation experiments, and this might be possible if a specific chemical abnormality in the blood, urine, or cerebrospinal fluid at a subclinical stage could be detected. Work so far has not given any sign of such a chemical indicator, but even if an

index was found in tissue from animals killed in the clinical stages this would be substantive evidence to the clinical and histological findings.

Finally, analyses of serum and tissue proteins are of assistance to the immunologists. Studies on proteins have not shown gross changes in serum or tissue, due partly to the complexity of the electrophoretic patterns obtained (Mould and Dawson, 1962), but in view of the observation of Kasting and Darcel (1963) that free amino acids are more abundant in scrapie than in normal brains and that hydrolysates of the proteins show different amino acid patterns, this work could probably be extended.

Chemical Analysis of body fluids and tissues

Early unpublished work by Stewart at the Moredun Institute showed that scrapie and normal sheep serum had similar contents of total protein, nonprotein nitrogen, and various inorganic ions. Recently, similar results have been found in analyses of total protein, albumin, globulins, sugar, cholesterol, urea, chloride, calcium, potassium, sodium, and phosphate (Avery, Mills, and Darcel, 1960; Darcel and Avery, 1960). On the basis of clinical and histological similarities between Wilson's disease and Casteigne-Lermitte's disease, copper and glycogen were measured in scrapie and normal sheep brains and shown to occupy the same concentration range in each group (Darcel, Avery, and Carson, 1961). Incidence of scrapie in sheep was not related to the haemoglobin or potassium types examined (Darcel and Avery, 1960).

Although there was no reason to expect any disease relationship between scrapie and β- globulin phenotypes in sheep, such typing could be useful as a genetic marker in questions of genetic inheritance of susceptibility and resistance. A survey of 50 sheep has been made, and anomalies did appear in the frequency of appearance of two types (unpublished).

In a paper chromatographic study of extracts of brain, urine, and plasma of normal and scrapie sheep, Kasting and Darcel (1963) found that acid-ethanol extracts of scrapie brain contained more ninhydrin-reacting material than normal brain, and that hydrolysates of the extracted residues showed a difference in amino acid patterns.

Millson, West, and Dew (1960) obtained similar negative results with blood and cerebro-spinal fluid constituents.

We have determined the total protein, nonprotein nitrogen (NPN), individual free amino acids, calcium, and magnesium in the serum of normal Cheviot sheep and of natural and experimental cases. In one experiment these constituents were measured in monthly samples from a group of 9 controls and 10 sheep inoculated with scrapie. Two sheep became infected, one after 5 months and the other after 7 months. Results were augmented by studying serum from 14 other clinical cases.

In an earlier experiment a detailed examination of the distribution of serum proteins in serial blood samples taken at monthly intervals from 15 sheep prior to and after inoculation was made. No change striking enough for diagnostic purposes was observed in the three cases of scrapie obtained. Particular attention was given to the early period after inoculation, but no changes were apparent until the late stages of clinical disease when any type of concurrent secondary infection could have been present.

Figure 1 shows that in normal, scrapie, and inoculated but symptomless sheep the values for protein, NPN, calcium, and magnesium occupy similar ranges in each group. In addition, the serial analyses gave similar fluctuations in all groups, with no trends being established even in the final stages of the disease.

The results of the amino acid analysis carried out by ion exchange chromatography are given in Figs. 2 and 3. Alanine, phenylalanine, and isoleucine appeared in lower concentrations in scrapie serum than in normal serum. Four other ninhydrin-reactive peaks from the ion exchange column also showed significant differences between the healthy and infected groups. Their concentrations are expressed in μmoles leucine, the reaction of which with ninhydrin was the colour standard for the procedure. "Pre-taurine" is a group of acidic substances eluted early from the column, of low concentration in serum, but scrapie serum always contained approximately double the quantity of normal serum. Urea is not satisfactorily determined by this

SERUM CONSTITUENT	NORMALS 9 SHEEP		INOCULATED BUT SYMPTOMLESS 8 SHEEP		SCRAPIES 16 SHEEP	
	Number of Samples	Mean ± S.D.	Number of Samples	Mean ± S.D.	Number of Samples	Mean ± S.D.
Total Protein (gm/100 ml)	98	7·19 ± 0·59	88	7·16 ± 0·23	16	6·98 ± 0·53
Non-Protein Nitrogen (mg/100 ml)	94	26·8 ± 5·1	84	25·6 ± 4·5	15	24·8 ± 5·0
Calcium (mg/100 ml)	83	10·2 ± 0·7	72	10·1 ± 0·7	4	9·70 ± 0·98
Magnesium (mg/100 ml)	83	2·40 ± 0·23	72	2·38 ± 0·27	4	2·10 ± 0·26

Figure 1.

AMINO ACID CONTENT OF SCRAPIE AND NON SCRAPIE SHEEP SERUM

(mg/100 ml)

Serum constituent	Non-scrapie 9 sheep		Scrapie 9 sheep		P	
	No. of samples	Mean ± S.D.	No. of samples	Mean ± S. D.		
Alanine	23	3.36 ± 0.49	9	2.85 ± 0.49	< 0.005	*
Aspartic acid	17	0.41 ± 0.19	9	0.29 ± 0.06		
Glutamic acid	23	3.00 ± 0.51	9	3.16 ± 1.18		
Glycine	23	4.59 ± 0.83	9	4.88 ± 0.87		
Isoleucine	21	1.71 ± 0.33	5	1.35 ± 0.51	< 0.05	*
Leucine	21	2.35 ± 0.49	5	2.05 ± 0.45		
Methionine	22	0.60 ± 0.22	9	0.58 ± 0.11		
Phenylalanine	17	1.19 ± 0.30	8	0.94 ± 0.14	< 0.05	*
Taurine	23	1.54 ± 0.39	9	1.71 ± 0.62		
Tyrosine	19	1.67 ± 0.41	9	1.45 ± 0.38		
Valine	22	3.42 ± 0.67	9	3.02 ± 0.60		

Figure 2.

OTHER NINHYDRIN-REACTING CONSTITUENTS
EXPRESSED AS MICRO-MOLES OF LEUCINE PER 100 ml OF SERUM

Serum constituent	Non-scrapie 9 sheep		Scrapie 9 sheep		p
	No. of samples	Mean ± S.D.	No. of samples	Mean ± S.D.	
"Pre-taurine"	17	2.6 ± 1.0	9	5.1 ± 1.2	< 0.001
Urea	23	12.6 ± 4.7	9	7.5 ± 3.6	< 0.010
$AspNH_2$-$GlutNH_2$-Ser-Threo	23	41.9 ± 7.0	9	30.6 ± 4.3	< 0.001
"X"	23	19.1 ± 5.3	9	14.5 ± 4.8	< 0.050

Figure 3.

technique, because of a low and variable colour yield with ninhydrin. The result with asparagine, glutamine, serine, and threonine (ASTG), which were not separated, is of interest since Kasting and Darcel found that threonine was absent from scrapie serum in the late stages of the disease although present in normal serum. The nine scrapie sheep quoted in figure 3 were all severe clinical cases. The compound "X" is possibly citrulline. The monthly analyses of the two experimental scrapie sheep did not show a marked trend in the concentration of alanine, phenylalanine, or isoleucine, the values remaining within the normal range, although in one case alanine and isoleucine fell sharply in the terminal stage. With "pre-taurine," urea, ASTG, and "X", abnormal values began to appear spasmodically about 3 months after inoculation and were only established in the latter phase of the disease.

Quantitative estimations have been made on the lipid content of sheep and goat brain tissue based on chloroform-methanol extraction, followed by a preliminary fractionation procedure (Folch and Lees, 1951). Useful data was obtained, but no significant differences between a scrapie-infected and scrapie-uninfected brain have been observed.

An analysis of phosphorus-containing brain constituents in terms of acid-soluble P, lipid P, protein-bound P, nucleic acids, and phosphoprotein was carried out on sections of brain tissue from normal and scrapie sheep. Small blocks (500 mg. to 1 g.) containing neuronal lesions, were cut from the thalmus, mesencephalon, pons, medulla, and cerebellum. Certain variations were observed but considerable verification is required with serial sectioning and histological examination of intermediate sections in order to establish the precise nature of the material examined (Mould and Slater, 1961).

Since the immunological response of nervous tissue is poor, methods of purifying and concentrating antigenic components might assist characterisation of the antigenic components and enhance the possible antibody reaction. Soluble proteins from sheep brain have been separated by electrophoretic and chromato-

graphic techniques, and antigenic components located by microimmunoelectrophoretic procedures (Mould and Dawson, 1962).

As part of a programme in the examination of cerebral-spinal fluid, involving serial sampling at two monthly intervals, from scrapie-inoculated goats, cholesterol content was estimated. Although the total cholesterol remained constant throughout in each of six scrapie-affected animals, the proportion of free to esterified cholesterol increased significantly at the time of slaughter. The histological condition of the brain varied from signs of very early lesions to severe scrapie lesions, and the change in cholesterol was observed in each case with a tendency toward a greater variation in the two more advanced cases with severe clinical symptoms.

Brain Tissue Enzymes in Scrapie

Succinic dehydrogenase and cytochrome oxidase.--The ideas behind the enzymic study of scrapie tissue have been mentioned previously. Succinic dehydrogenase and cytochrome oxidase were also studied as mitochondrial markers in relation to the centrifugal fractionation methods discussed elsewhere. A fairly full general study has been made of these enzymes in mouse and goat brain.

The manometric method of Schneider and Potter (1943) was used. This involves measurement of oxygen uptake in presence of succinate or ascorbate plus cytochrome c, and results are expressed as μl oxygen absorbed per 100 mg. tissue per hour at 37°C. The determinations of total protein in normal and scrapie brains showed no significant differences either for goats or mice.

Figure 4 contains the results of succinic dehydrogenase and cytochrome oxidase determinations in normal (3- to 6-month-old) mice and mice infected with Suffolk-strain mouse scrapie. Differences between the healthy and diseased groups are not significant, and even in very severe cases these enzyme activities fell within the normal range. This result is of interest in relation to the finding of high scrapie activity of mitochondrial-rich fractions in brain homogenates (Hunter, Millson, and Meek, 1963), and indicates that if the agent is attached to the mitochondria in any way, then the function of these particles is not severely affected by this association.

Brain material for the goat work was made available from a pilot titration experiment in which six groups of five goats were inoculated with saline or dilutions of scrapie brain homogenate. All the goats were killed during one week, and enzymic estimations carried out on

	NORMAL MOUSE BRAIN		SCRAPIE MOUSE BRAIN	
	Number of Samples	Mean ± S.D	Number of Samples	Mean ± S D
Protein Content (mg./100mg brain)	21	10·9 ± 0·7	22	11·0 ± 0 9
Succinic Dehydrogenase (μl O_2/hr/100mg. brain)	30	790 ± 83	22	800 ± 64
Cytochrome Oxidase (μl. O_2/hr/100 mg. brain)	19	7120 ± 1120	12	7270 ± 1700

Figure 4.

fresh parietal gray matter from transverse slices of the occipital lobe at the level of the hippocampus (Barlow, 1963). Results are quoted in Figure 5., along with an additional analysis on another normal goat brain not in the titration experiment.

Of the goats that received scrapie inoculum, the 10^{-1} group had the highest SDH and cytochrome oxidase levels and this trend was reflected back through the dilutions to 10^{-5} with cytochrome oxidase, although not with succinic dehydrogenase. Two of the control brains, however, exhibited very high cytochrome oxidase activities, close to the values for the 10^{-1} samples. These results must, therefore, be treated with reserve, although faulty dissection of the gray matter is not thought to have been the cause of the rather erratic values, since determinations on adjacent slices generally showed agreement to about 10 percent.

In Figure 6 are collected the SDH and protein contents of sediments from differential centrifugation on homogenates of four normal and four scrapie mouse brains. The recoveries of SDH activity and protein were good, and these homogenates all showed similar sedimentation properties. This is an important factor in relating the scrapie agent to specific cellular components.

Esterases.--The distribution of esterases has been studied by starch and acrylamide gel electrophoresis of high-speed supernatants of homogenates of mouse brain, spleen, and liver and of goat spleen followed by incubation of the gels with naphthyl acetate and naphthol coupling dye. Visual inspection of the complex patterns failed to show any gross differences between the esterase constitution of scrapie and normal tissue extracts.

Hydrolytic enzymes.--Allison and Sandelin (1963) have found increased levels of several hydrolytic enzymes in the livers of mice infected with hepatitis virus and have discussed the possible effects of viral disease on lysosomes, the particles which are thought to contain several hydrolases. Kovacs et al (1960) also found elevated activity of hydrolases in poliomyelitis virus- HeLa cell systems, but, in the main, papers in this field relate to nucleic acid metabolism. Little is at present known of the lysosomes of brain, but they may be relatively stable structures compared with those of liver.

Our first studies have been on the acid phosphatase of normal and scrapie brain. No elevation of activity has as yet been found, even in very severe cases. In relation to prolonged treatment at pH5, brain lysosomes

DILUTION	CASES	BRAIN PROTEIN CONTENT (mg/100mg) Mean ± SD	SUCCINIC DEHYDROGENASE (µl/hr/100mg) Mean ± SD	CYTOCHROME OXIDASE (µl O_2/hr/100mg) Mean ± SD
CONTROL	5/5	101 ± 01	482 ± 47	3174 ± 1604
10^{-9}	4/4	100 ± 04	440 ± 14	1978 ± 536
10^{-7}	3/3	98 ± 02	442 ± 14	1690 ± 493
10^{-6}	2/5	98 ± 02	433 ± 9	2850 ± 170
10^{-5}	4/5	100 ± 03	444 ± 25	2901 ± 392
10^{-4}	4/5	104 ± 04	525 ± 4	4733 ± 710
NGB 7297	+	102	486	1848

Figure 5.

FRACTION	g × min	PROTEIN				SUCCINIC DEHYDROGENASE			
		NORMAL		SCRAPIE		NORMAL		SCRAPIE	
		Protein Recovered Average %	% of Recovered Protein in Fractions	Protein Recovered Average %	% of Recovered Protein in Fractions	SDH Recovered Average %	% of Recovered SDH in Fractions	SDH Recovered Average %	% of Recovered SDH in Fractions
N	1000 × 10		12 2		17 7		4 6		6 5
M_1	3000 × 10		28 1		25 0		62 2		57 9
M_2	10000 × 10	83 7	17 4	95 6	13 9	93 9	29 4	92 5	27 5
M_3	30000 × 10		11 0		11 6		4 4		8 1
R	30000 × 35		5 9		7 3		-		-
S	Final Supernatant		25 3		24 3		-		-

Figure 6.

appear to be more stable than those of liver.

Proteases are also thought to be components of lysosomes, and these should be examined soon, in view of the interesting finding of Kasting and Darcel that scrapie sheep brain may contain more free amino acids than normal and that the hydrolysates of brain protein exhibit different amino acid patterns.

Figure 7 shows that the weights of scrapie mouse brains are much lower than those of normal brains. This may be due to some quite separate effect, such as loss of water caused by altered ionic distribution, but dry matter measurements do not bear this out. It is tempting to speculate that lysosomes play a role in the disease process by hydrolysing protein and causing a loss of dry matter from the brain.

In conclusion, certain directions of this work can be summarised and extended. On present evidence there appears little reason to doubt that the causal agent of scrapie is of a transmissible virus-like nature. Quantitative work on the larger scale now possible in small

	NORMAL		SCRAPIE	
	Number of Samples	Mean ± SD	Number of Samples	Mean ± SD
Brain Weight (mg)	87	433 ± 33	85	401 ± 40

Figure 7.

animals should lead progressively to a more precise understanding of its location and morphology. Its main peculiarity can be considered to reside in its marked association and binding to tissue. The lack of success in detection by electron microscopy, except in relation to the particles present in sheep brain, at this stage is significant and not at variance with the general experimental evidence. With the possibility of producing more purified and concentrated preparations of known biological activity in the mouse, the

chances of establishing unequivocally the presence or absence of characteristic particles is much increased.

There is evidence to suggest that the agent may exist under different conditions according to the host species and tissue. This is not unusual in viral diseases but must be considered in relation to the special response of the disease in sheep, and investigation of sheep tissue should not be neglected in favour of the mouse. This could form a basis for speculation on the spread of natural infection and the apparent resistance of a high proportion of a sheep population to natural or experimental scrapie. Thinking in terms of a conventional virus, the production of purified and concentrated preparations of the scrapie agent could provide potent antigenic material for further serological work.

Biochemical investigations have been almost entirely carried out on brain tissue. Subcutaneous or intraperitoneal inoculation, however, induce the identical final condition of neuronal damage as that by the intracerebral route, and the possibility that in the latter case the agent undergoes an intermediate stage in another tissue organ before returning at a later stage to the brain must not be forgotten.

REFERENCES

Allison, A.C., and Sandelin, K. (1963), Jour. Expt. Med. 117; 879.

Avery, R.J., Mills, J.A., and Darcel, C.le Q. (1960), Canad. Jour. Comp. Med., 24: 241.

Bachrach, H.L., and Schwerdt, C.E. (1952), Jour. Immunol. 69: 551.

Barlow, R.M. (1963), Jour. comp. Path. 73: 61.

Bradley, P.L., (1957), Nature, London 180: 1418.

Campbell, A.M.G., Norman, R.M., and Sandry, R.J. (1963), Jour. Neurol. Neurosurg. Psychiat. 26: 439

Chandler, R.L. (1961), Lancet, 1: 1378.

Chandler, R.L. (1963), Res. Vet. Sci. 4: 276.

Daems, W.Th., and Van Rijssel, Th.G. (1961), Jour. Ultrastruct. Res. 5: 263.

Darcel, C.le Q., and Avery, R.J. (1960), Canad. Jour. Comp. Med. 24: 17.

Darcel, C.le Q., Avery, R. J., and Carson, R.B. (1961), Nature, London 189: 410.

Eklund, C.M., Hadlow, W.J., and Kennedy, R.C. (1963), Proc. Soc. Expt. Biol. Med. 112: 974.

Field, E.J., Miller, H., and Russell, D.S., (1962), Jour. Clin. Path., 15: 278.

Folch, J. and Lees, M. (1951), Jour. Biol. Chem., 191: 807.

Hunter, G.D., Millson, G.C., and Meek, G. (1963), Jour. Gen. Microbiol.

Kasting, R., and Darcel, C.le Q. (1963), Res. Vet. Sci. 4: 518.

Kovacs, E., Wagner, G., and Sturtz, V. (1960), Z. Naturforsch. 156: 506.

Millson, G.C., West, L.C., and Dew, Sally M. (1960), Jour. Comp. Path. 70: 194.

Mould, D.L., and Dawson, A.McL., (1962), Biochem. Jour. 84: 52P.

Mould, D.L., and Slater, J.S. (1961), Proc. V. Internatl. Cong. Biochem, Moscow, p. 217.

Mould, D.L., and Smith, W. (1962 a), Jour. Comp. Path. 72: 97.

Mould, D.L., and Smith, W. (1962 b), Jour. Comp. Path. 72: 106.

Mould, D.L., Smith, W., and Dawson, A. McL. (1963), Biochem. Jour. 87: 24P.

Novikoff, A.B. (1961), The Cell, vol. 2, p. 423. Ed. Brachet, J. and Mirsky, A.E., New York: Academic Press.

Schneider, W.C., and Potter, V.R. (1943), Jour. Biol. Chem. 149: 217.

Taverne, J., Marshall, J.H., and Fulton, F. (1958), Jour. Gen. Microbiol. 19: 451.

Whittaker, V.P. (1959), Biochem. Jour. 72: 694.

Zlotnik, I., and Rennie, J.C. (1963), Jour. Comp. Path. 73: 150.

DISCUSSION

Dr. Stamp:

It is possible that this agent, as Dr. Gordon pointed out, may be attached to some particle, and it may be necessary to break the agent away from the particle before we get any further. Most certainly it is very disappointing that we in these active preparations have been unable to see anything with the electron microscope which remotely resembles a virus unit.

I think, Dr. Gordon, we have been doing this longer than you, and we are still searching.

Dr. Morris:

Did you examine any of the concentrates of activity to see whether it is antigen or not?

Dr. Stamp:

I haven't written a paper on this, but we have done a considerable amount of serological work using these concentrates.

Dr. Morris:

Promising or negative?

Dr. Stamp:

Nothing, nothing at all. We are not apparently concentrating the agent and I would emphasize this.

Dr. Morris:

Tenfold?

Dr. Stamp:

Perhaps

Dr. Burger:

First of all, where you purified fractions, were they tested for nucleic acid content? Secondly, could the biological activity be inhibited by any of the nucleic acids?

Dr. Stamp:

I was rather worried that this question would be asked. I rather avoided it in my paper. We have done some work, and some further work is being done at the present time using the deoxy and ribonucleases to try to see if they have any activity on the scrapie agent. Up to the present time, we have not been successful in demonstrating activity. But I would emphasize that until the mice came along as experimental animals, this was an exceedingly difficult problem. We were using sheep for biological assay and to do titrations in the numbers that we wanted to do was impossible for every time you do a titration in the sheep, it means using 30 sheep for each dilution and you can't do many titrations at that rate.

Even at the end of the day one was never quite sure what the titration meant. So that really until the mice came along, we were pretty lost, and we are only now going on with this type of work.

Up to the moment, therefore, I have nothing to report, but what little work we have done has not been very encouraging. We think that there may be something protecting the nucleic acid. Have you been doing this at Bethesda? Are you getting anywhere?

Dr. Gibbs:

Not yet.

Dr. Mayall:

I can confirm the same, too.

TISSUE CULTURE STUDIES ON SCRAPIE

J. G. Brotherston, J. T. Vantsis, J. M. K. Mackay, and A. Drysdale[1]
(Presented by J. T. Stamp[2])

INTRODUCTION

In the initial approach to the problem of growing scrapie agent in tissue culture the following methods were adopted:

(1) Outgrowth of tissues from animals affected at various stages were compared with similar cultures of normal animals; a wide range of tissues from sheep and goats were examined.

(2) Normal adult and foetal sheep and goat tissues were used as substrates and inoculated with scrapie material.

(3) Line cells were obtained from other laboratories and also developed from foetal goat tissues. These were then examined for abnormal growth after contact with the scrapie agent.

(4) Attempts were made to demonstrate inhibition of cell metabolism by the scrapie agent and also to demonstrate the interference phenomenon with other viruses. Concentration and/or extraction of the scrapie agent from tissues for tissue culture purposes were also made.

These studies have given no evidence of cytopathogenicity or any consistent departure from the normal even when cultures were observed for periods up to many weeks. The investigations were made with the scrapie agent of the Cheviot line and of a goat-adapted line, both being available as biologically tested pool materials. Both were derived from the same source, namely, the Wilson strain of Cheviot agent which originated at Moredun. At the time it was only possible to test material for infectivity by using goats or sheep and, although some tissue culture series were tested, it was not practicable to do this routinely or extensively.

When the scrapie agent was passed in mice (Chandler, 1961) these animals could for obvious reasons be freely available both as donors and indicators of the agent, so that more extensive testing of tissue culture systems became possible.

MOUSE-ADAPTED SCRAPIE AGENT IN TISSUE CULTURE

Mouse Spleen Plasma-Clot Cultures

Spleen fragment cultures from mice infected with a third-mouse-passage of goat scrapie (drowsy type) were initiated from mice showing signs typical of mouse scrapie. These were maintained with regular changes of medium, aliquots of supernatant fluid after disruption of the cells were harvested at intervals and injected into mice. It was shown that the agent survived for at least 16 days in cultures of this type. In another experiment 1 of 10 mice developed scrapie lesions following intracranial inoculation with the harvest from a 28-day spleen culture. In both experiments the presence of calf serum appeared to be inhibitory as compared with horse serum.

Primary Embryo Mouse Tissue Culture as Substrate

These cultures are of mixed cells, but fibroblasts predominate and they can be maintained for up to 2 months. Primary and secondary cultures of scrapie (drowsy type) in such tissue for varying periods of time were carried out, the material being tested in mice

[1] Microbiology Department, Animal Diseases Research Association, Moredun Institute, Edinburgh, Scotland.
[2] Director, Animal Diseases Research Association, Moredun Institute, Edinburgh, Scotland.

for infectivity. The data so far obtained is as follows: -

(a) With cell-free extracts of infected mouse brain as inoculum, the agent survived up to 48 days in cultures incubated at 32°C. and at 37°C. The medium was changed every 4 to 5 days.

(b) The scrapie agent survived in subcultures of (a) made at 39 days, the secondary culture having been incubated for a further 19 days. Subsequent subcultures were negative.

(c) Spleen extracts from mice, whose brains were positive for scrapie, were cultured as for (a) and (b). When tested in mice the cultures failed to produce scrapie in mice after 10½ months' incubation.

(d) Extracts of infected mouse brain and spleen have not produced a cytopathogenic effect (C.P.E.).

Miscellaneous Cultures

Another agent isolated from fifth-passage mouse-adapted Suffolk sheep scrapie in primary pig kidney is currently under investigation.

DIRECT TISSUE CULTURE OF MATERIAL FROM NATURAL CASES OF SCRAPIE IN THE SHEEP AND GOAT

Unsuccessful attempts to culture agents from scrapie material have been made from material which originated as passage material from Cheviot sheep, sheep scrapie adapted to goats, or goat scrapie adapted to mice. However, routine cultures of brain tissue from a number of field cases of the disease have been carried out on primary pig kidney cultures, and cytopathogenic agents have been isolated and passaged in tissue culture.

The following details regarding the material cultured are given.

SB 72. Brain from a 3-year-old half-bred sheep, which was one of three sent to the diagnostic laboratory in Edinburgh with symptoms of acute encephalitis, was cultured. Neither a virus nor bacterial agent was recovered on primary culture or by mouse inoculation. Brain sections examined some weeks later showed that all three sheep had lesions characteristic of scrapie. Further tissue cultures and mouse inoculations were subsequently made, using brain stored at -20°C. as inoculum.

GB 7304. Tissues from a goat, which had developed scrapie without inoculation, were cultured. The goat had been in contact with goats inoculated with scrapie (Mackay and Smith, 1961).

SB 757. Tissues from a Suffolk sheep sent to Moredun in the terminal stages of scrapie disease.

The following is the method of tissue culture which was used:

Monolayers of secondary pig kidney cells in test tubes with "flying" coverslips were prepared in the conventional manner. The nutrient fluid was Hank's lactalbumen hydrolysate with 10 percent calf serum, with 100 units of penicillin and 50 units streptomycin per ml. For initial infection, 10 percent of the source brain was emulsified in Hank's and lightly centrifuged, the supernate was emulsified and placed in contact with the monolayers in stationary tubes for 1 hour at 37°C. The inoculum was then removed and replaced with nutrient fluid. Cultures were maintained for 7 days before passage, but if a C.P.E. was noted on examination of a sample coverslip stained with Giemsa the cultures were subcultured earlier. For passage of cell cultures, tubes were frozen and thawed three times, using dry ice and acetone, and an aliquot of the whole culture was then placed in contact with new monolayers in tubes. In the first instance no attempt was made to pass cell-free supernatant fluid. For further use whole cultures were frozen and stored at -20°C. and, on occasion, freeze-dried after each passage.

RESULTS

Each of the three source materials caused a similar C.P.E. after two to five passages, but most subsequent examinations with the exception of the neutralisation tests have been carried out with SB 72 material. In the first instance, it was noted that some cells were detached from the coverslips at 5 to 7 days but the remaining cells appeared healthy. After

the fifth passage this effect was noted at 48 hours. Examination of coverslips between 6 and 24 hours as fresh preparations under phase contrast showed cells that were grossly vacuolated and with bubblelike processes extending outward from the cell wall. Occasionally refractile material was noted in the vacuoles or in the cell cytoplasm adjacent to the processes. The cell nucleus was often distorted by the presence of the vacuoles. In figure 1 a photograph of the complete C.P.E. on a culture is shown, but the effect is more characteristic if cells detached from the glass are examined. Studies on the cytopathogenicity have not been completed, but the effects are remarkably similar to the morphological changes that cause no loss of viability in Ehrlich ascites tumour cells after incubation with 0.0096 percent solution of formaldehyde (3×10^{-3} M) (Ratzenhofer and Zangger, 1963).

Virus Titre

The agent reproduced the effect to titres of 10^{-6} to 10^{-7}, and this has been maintained over 35 passages. Passage can now be made with cell-free supernate after 48 hours of culture.

Heat Resistance

Affected pig kidney cells in the presence of 10 percent calf serum were heated at 80°C. for 20 minutes, and the titre was reduced from 10^{-7} to 10^{-2} per ml. of the cell-free supernate. After heating at 100°C. for 10 minutes, the agent survived in one out of three trials. In serum-free medium, the agent did not survive heating to 100°C.

Resistance to Ether

After treatment with 20 percent ether at 4°C. for 15 hours, the agent could be passed in culture. No titration has been carried out.

Resistance to Freezing and Drying

The agent can be stored at -20°C. for at least 4 weeks and survives freeze-drying. No potency tests have been made.

Growth on Other Tissues

No evidence of cytopathogenicity has been found in embryo mouse tissue culture, mouse spleen macrophages, sheep kidney, calf kidney, or BHK 21 inoculated with pig kidney tissue culture passages of SB 72. A pig line cell (STICE) similarly inoculated has shown C.P.E.

Neutralisation Tests

Antiserum against SB 72, prepared in rabbits, neutralised 100 TCD_{50} of homologous agent to titre of 1:60. Cross-neutralisation tests with GB 7304 and SB 757 indicated that all three agents had common antigens. Sera prepared from noninfected pig kidney cells did not neutralise the effect.

Antiserum prepared against T 80 and V 13 pig enteric viruses did not neutralise SB 72, but slight neutralisation was obtained with antiserum to Talfan virus.

Electron Microscope Studies

Electron microscope studies with crude supernates of SB 72 of 30th passage have demonstrated hexagonal bodies of about 25 mμ in size with subunits (see fig. 2).

Passage in Mice

A 10 percent brain supernate of the original sheep brain SB 72, when injected intraperitoneally into sucking mice, killed the mice in 18 days. This effect could not be repeated by further passage of brains or spleens of moribund mice, but the same cytopathic agent as described above was recovered in tissue culture from spleen and brain. The 10 percent brain emulsion of SB 72 does not affect weaned mice, as yet, when injected intraperitoneally or intracranially.

Pathogenicity for Mice and Sheep

The following animal inoculation experiments are in progress but at present no signs of pathogenicity have been noted. Inoculation was by the subcutaneous and intracranial routes.

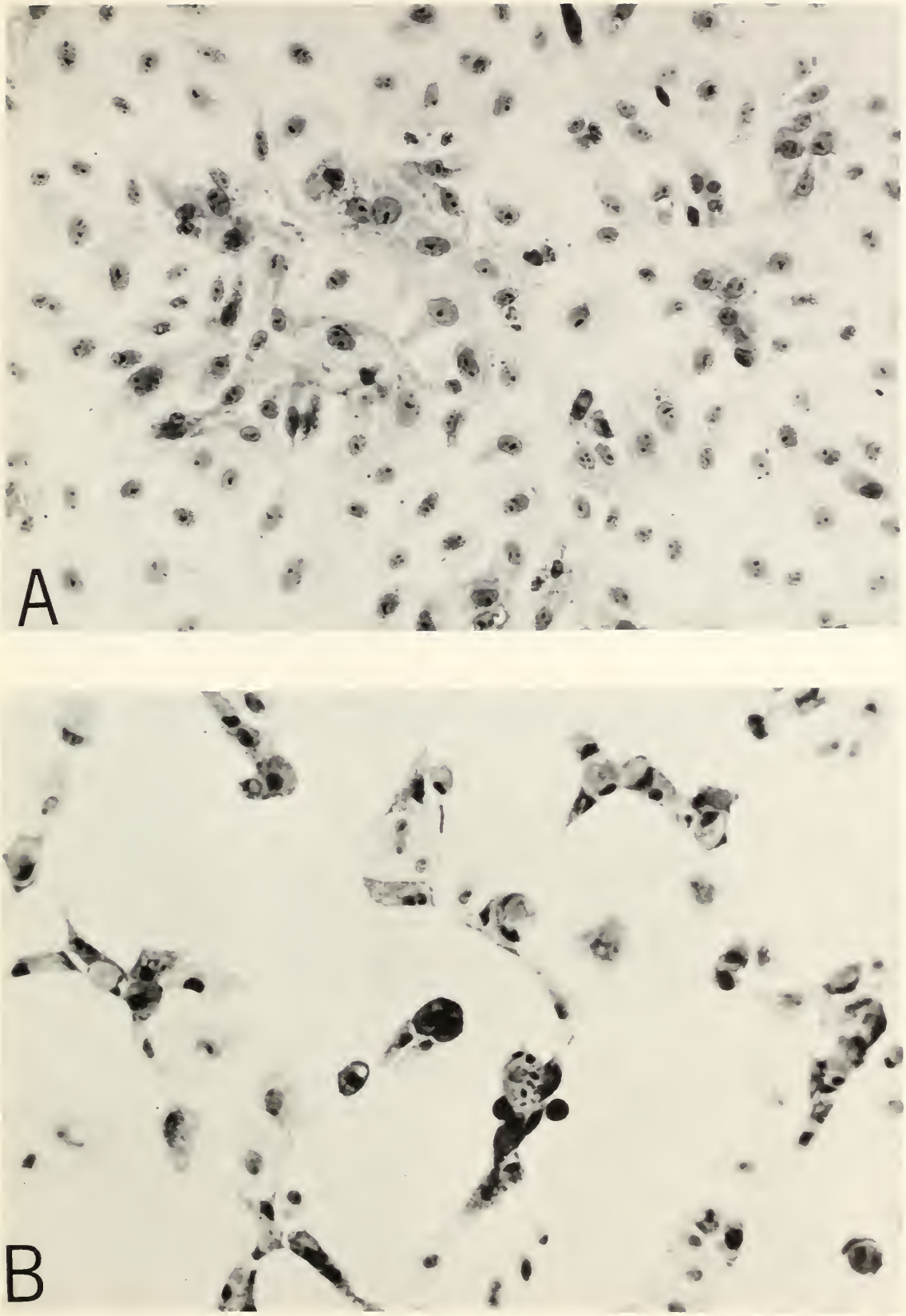


Figure 1.--A, normal pig kidney monolayer culture (X 220); B, SB/72 on pig kidney monolayer culture after 24 hours (X 220).

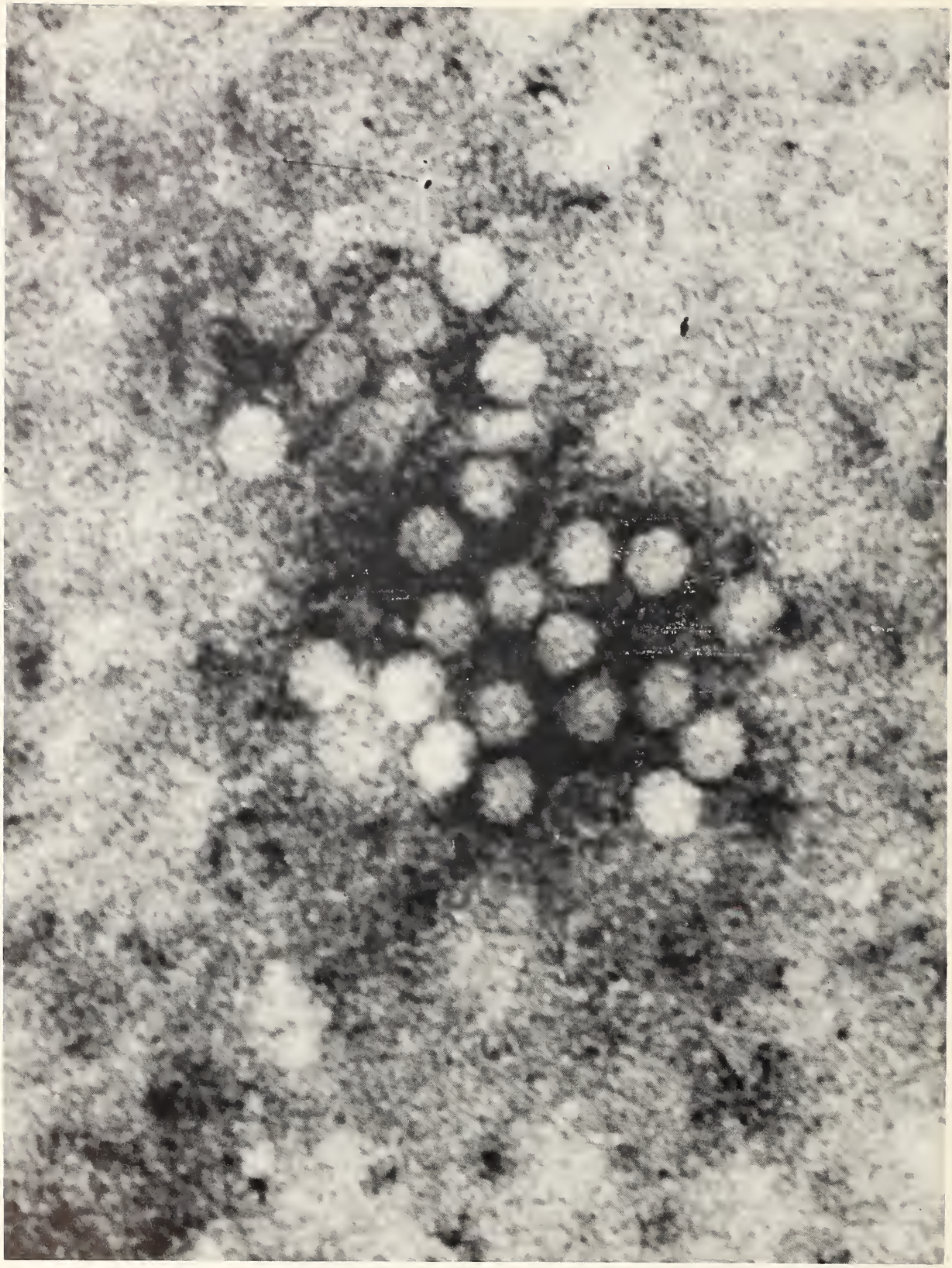


Figure 2.--SB 72 electron micrograph X 400,000.

Agent	Passage	Time after injection (January 1964)	
		Mice	Sheep
SB 72	10 percent sheep brain	Months 12	Not done
	Tissue culture 2d pass	11	9 months
	Tissue culture 10th pass	8	7 months
	Tissue culture 20th pass	5	Not done

DISCUSSION

Since these cytopathogenic agents were isolated, the real point at issue is whether one or all of these were initiated from the brains of scrapied sheep and goats or from the pig kidney used as substrate. The reproduction of typical scrapie by animal inoculation of the agent would be strong evidence for the former, but attempted classification of the agent as a pig virus is also being carried out.

Mouse scrapie agent has been shown to survive in tissue culture systems for up to 48 days and the ability to infect mice with scrapie has survived one subculture. This perhaps constitutes grounds for optimism that the scrapie agent may be able to multiply in tissue culture systems.

It is too early to rule out the possibility of a positive transmission of scrapie to mice or sheep, using tissue culture passage material of sheep brain SB 72, for it must be borne in mind that sheep scrapie agent on transmission to a new host may have an extended incubation period. For example, Cheviot agent via Welsh mountain sheep required 15 months to produce the disease in goats (Pattison, 1957; Gordon and Pattison, 1957). Suffolk sheep agent in mice caused a symptomless infection on first passage, requiring 12 to 15 months before histopathological lesions were observed (Zlotnik and Rennie, 1962), while transmission of scrapie to rats was protracted and symptomless in first passage (Chandler and Fisher, 1963). On these grounds it is not unreasonable to expect an agent grown on pig tissues to exhibit similar characteristics when returned to another species. For these reasons, the cytopathogenic agent obtained from scrapied mice that might be expected to reproduce the disease in mice more readily than a sheep or goat isolate is being further investigated.

REFERENCES

Chandler, R. L. (1961) Lancet 1: 1378.
Chandler, R. L., and Fisher, Jacqueline (1963) Lancet 2: 1165.
Gordon, W. S., and Pattison, I. H. (1957). Vet. Rec. 69: 1444.
Mackay, J. M. K., and Smith, W. (1961) Vet. Rec. 73: 394.
Pattison, I. H. (1957) Lancet 272: 104.
Ratzenhofer, M. and Zangger, J. (1963) Nature 200: 184.
Zlotnik, I. and Rennie, J. (1962) Jour Comp. Path. 72: 360.

DISCUSSION

Dr. Morris:

What is the appearance of the pig agent?

Dr. Stamp:

There have never been any satisfactory photographs of the Talfan agent, unfortunately. There don't seem to be any. This is our doubt. I am not trying to say we have got scrapie virus. If it is Talfan virus, it must be common in the land.

Dr. Gibbs:

Was Talfan virus neutralized by your serum?

Dr. Stamp:

No. Only the one-way cross.

Question:

What would be the particle size?

Dr. Stamp:

The particle size is very small; somewhere in the region of 20 millimicrons. A very small particle about the same size as the Talfan virus.

EVOLUTION OF SCRAPIE VIRUS INFECTION IN MICE

C. M. Eklund, R. C. Kennedy, and W. J. Hadlow[1]
(Presented by W. J. Hadlow)

The prolonged incubation period, slowly progressive clinical course, and noninflammatory lesions of scrapie suggest that the underlying infectious process is radically different from that implicated in the usual acute viral disease. Yet, despite much recent investigation (1),[2] little is known about the characteristics of scrapie virus infection in either the natural host, the sheep, or the usual experimental hosts, the goat and the mouse. An intelligent study of this slow infection requires detailed knowledge of the sequential and quantitative development of the virus in infected animals and the correlation of this information with the occurrence of clinical signs and histologic changes. The demonstration by Chandler (2) that scrapie virus causes a protracted neurologic disease in the mouse has provided a convenient experimental model for studying this slow viral infection.

The purpose of this study was to obtain quantitative data on the temporal distribution of scrapie virus in inoculated mice and a clearer picture of the development of histologic changes in relation to clinical disease. Accordingly, young mice were inoculated subcutaneously with the virus and at intervals thereafter were killed for histologic examination and for detection of virus in various organs. The study has not been completed, but the principal observations are presented here.

[1] Medical Director, Insect-Borne Diseases, Research Microbiologist, Insect-Borne Diseases, and Research Veterinarian, Pathology and Serology, respectively, Rocky Mountain Laboratory, National Institute for Allergy and Infectious Diseases, Public Health Service, U.S. Department of Health, Education and Welfare, Hamilton, Mont.

[2] Figures in parentheses refer to references at end of paper.

MATERIALS AND METHODS

Swiss mice of the Rocky Mountain Laboratory stock were used when 21 days old. Scrapie virus used represented the 4th mouse brain passage after 8 serial intracerebral passages in goats. While under ether anesthesia, 140 mice were inoculated subcutaneously in the left thigh with 0.05 ml. of a 10^{-2} dilution of virus. As determined by intracerebral inoculation of young mice, the titer of this viral suspension was $10^{-7.5}$. Six mice, taken at random, were killed at 1, 2, and 4 weeks after inoculation, and then at 4-week intervals until the experiment was completed. Three were used for histologic examination. Tissues from the other three were pooled and weighed for detection of virus. Serial ten-fold dilutions through 10^{-8} were made in 0.85 percent solution of NaCl containing 10 percent normal rabbit serum. Each dilution, in 0.03 ml. amounts, was injected intracerebrally into six mice. The mice were observed for 1 year. The presence of virus was based on the occurrence of a typical pattern of fatal progressive disease in inoculated mice.

RESULTS

Distribution of Virus

The essential data are summarized in table 1. Virus was detected first in lymphocytic tissues. One week after inoculation of the mice, virus was found in a 10^{-4} dilution of spleen. Virus was not detected in any tissue at the second week, but at the fourth week it was detected in a 10^{-3} dilution of spleen and of peripheral lymph nodes (axillary and inguinal). From then on, virus was consistently present in these two tissues. At the 16th and 20th

Table 1.--Temporal distribution and amount of scrapie virus in mice inoculated subcutaneously

Organ[1]	Weeks After Inoculation											
	1	2	4	8	12	16	20	24	28	28[3]	32[3]	36[3]
Spleen	[2]10^{-4}		10^{-3}	10^{-6}	10^{-6}	10^{-7}	10^{-7}	10^{-5}	10^{-6}	10^{-5}	10^{-6}	(4)
Lymph Nodes			10^{-3}	10^{-6}	10^{-5}	10^{-5}	10^{-5}	10^{-4}	10^{-5}	10^{-4}	10^{-5}	(4)
Submaxillary salivary gland				10^{-6}	10^{-5}	10^{-6}	10^{-6}	10^{-6}	10^{-7}	10^{-7}	10^{-5}	
Thymus				10^{-4}	10^{-5}	10^{-5}	10^{-5}	10^{-5}	10^{-4}	10^{-1}	10^{-5}	(4)
Lung					10^{-3}	10^{-3}	10^{-3}	10^{-4}	10^{-2}	10^{-2}		
Gut					10^{-3}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-4}	10^{-3}	(4)
Spinal Cord					10^{-1}	10^{-5}	10^{-5}	10^{-7}	10^{-6}	10^{-7}	10^{-8}	(4)
Brain						10^{-4}	10^{-3}	10^{-6}	10^{-7}	10^{-7}	10^{-6}	(4)
Bone marrow (femur)								10^{-1}	10^{-3}	10^{-4}	10^{-2}	
Liver									10^{-1}	10^{-1}	10^{-1}	
Uterus							10^{-1}			10^{-2}		

[1] Pooled from 3 mice.
[2] Highest dilution in which virus was detected.
[3] Clinical signs present.
[4] Virus detected in 10^{-1} dilution, the only one tested. Titration to be completed.

weeks, virus was present in a 10^{-7} dilution of spleen, the highest attained. Virus was detected in a 10^{-6} dilution of lymph nodes at the 8th week. Thereafter, it was consistently present usually in the 10^{-5} or 10^{-4} dilution.

Eight weeks after the mice were inoculated, virus appeared in the thymus and submaxillary salivary glands. The highest dilution of thymus in which virus was found, 10^{-5}, occurred at the 12th week; this was the usual dilution in which virus was found thereafter. In the salivary glands, virus was usually present in the 10^{-6} or 10^{-5} dilution, but occurred in the 10^{-7} dilution, the highest reached, at the 28th week.

At the 12th week, virus was found in the lung, intestine (upper colon and lower ileum, and spinal cord. Usually, virus was detected in the 10^{-2} or 10^{-3} dilutions of lung; the highest reached was the 10^{-4} dilution at 24 weeks. In intestinal tissue, virus was usually found in the 10^{-3} or 10^{-4} dilution. The highest dilution in which virus was detected was 10^{-5} at the 28th week.

In the spinal cord, virus was detected only in the 10^{-1} dilution at the 12th week, but by the 16th week it was present in the 10^{-5} dilution; this gradually increased to 10^{-7} at the 28th week and 10^{-8} at the 32d week. Interestingly, at the 28th week, virus was detected in a 10^{-6} dilution of spinal cords from a healthy-appearing group of mice and in a 10^{-7} dilution of cords from definitely sick mice.

Virus was not detected in the brain until the 16th week, when it appeared in the 10^{-4} dilution. The amount of virus gradually increased to the 28th week, when it appeared in the 10^{-7} dilution in healthy as well as sick mice. At the 32d week, when the mice examined were sick, virus appeared in the 10^{-6} dilution. At the 36th week, when again the mice examined were sick, virus was found in the 10^{-1} dilution (the only tested) of brain and spinal cord as well as in spleen, lymph nodes, and thymus. The additional dilutions of these tissues are now being tested.

Virus was detected in a 10^{-1} dilution of bone marrow (femur) at the 24th week; it was constantly present thereafter, though never in a high dilution. Virus was detected also in a 10^{-1} dilution of liver at the 28th through the 32d week. On two occasions, a small amount of virus appeared in the uterus. At no time, however, was virus found in blood clot, serum, testis, or kidney.

Pattern of Disease

Some information was obtained also about the individual susceptibility of the 140 mice inoculated subcutaneously with the 10^{-2} dilution of virus on September 7, 1962. The first sick mouse was observed February 15, 1963; the last two that became sick were observed about October 15, 1963. At the time the remaining mice were discarded in December 1963, two were still apparently healthy. Although mice became sick over a period of 8 months, most of them were sick or dead by the end of May 1963, i.e., during a period of about 3 months.

Histologic Findings

Particularly early in the evolution of clinical scrapie in the mice, astrocytosis (increase in size and number of astrocytes) was the most easily recognized histologic alteration in the central nervous system. This change, which seemed to coincide with onset of clinical signs, was observed first in the spinal cord. Overt changes in neurons were not apparent early in the disease.

COMMENT AND SUMMARY

In young mice inoculated subcutaneously, scrapie virus first appeared in the spleen and peripheral lymph nodes, where it persisted to the time of death of the mouse. Soon after its appearance in these tissues, virus appeared in the thymus and in the submaxillary salivary glands. This early predilection of the virus for tissues important in the defense against infection may be related to the further progression of the disease. Apparently, the mouse has no effective defense against scrapie virus.

After slow replication in lymphocytic tissues, the virus spread to other structures, such as the lung and intestines, during the long incubation period. Here, virus persisted in fairly low dilutions.

Virus first appeared in the central nervous system relatively late in the course of the infection. After the 16th week, the amount of virus gradually increased to the 28th week, when equally high titers were found in healthy as well as sick mice. Obviously, no direct relation exists between the amount of virus in the central nervous system and the occurrence of overt clinical disease. It seems likely that the appearance of clinical disease is more closely related to the length of time virus is present in the central nervous system than to any specific amount of virus present in the brain and spinal cord.

The occurrence of large amounts of virus in the submaxillary salivary glands is obviously important in any consideration of the mechanism by which scrapie virus spreads in nature. Elimination of virus from the respiratory tract and intestinal tract also must be considered, though it may be less significant.

The data obtained in this study suggest several explanations for the pathogenesis of scrapie: 1. Involvement of tissues critical in the defense mechanism of the body, such as the spleen, lymph nodes, and thymus, may inhibit the formation of protective antibodies and so permit the continued replication of virus and its subsequent spread to the central nervous system where destructive changes lead to death, 2. The slow production of virus may result in a state of tolerance so that the usual defense mechanisms are not set in motion, 3. The chemical structure of the surface of the virus particle may be such as not to stimulate the immune mechanism of the body. Most likely many factors are important in causing the fatal, progressive but protracted course of the disease.

REFERENCES

1. Stamp, J.T. Scrapie: a transmissible disease of sheep. Vet. Rec. 74: 357-362. 1962.
2. Chandler, R.L. Encephalopathy in mice produced with scrapie brain material. Lancet 1: 1378-1379. 1961.

DISCUSSION

Dr. Hadlow:

I would like to call on my colleague, Dr. Carl Eklund, medical virologist, for comment as it represents largely his work.

Dr. Eklund:

I have been a little confused listening for the last few days. To me, scrapie is simply another virus disease. The agent is a particulate thing. It isn't larger than 50 millimicrons.

We use exactly the same techniques that we use in the virus field generally. And I don't think that a person should be too impressed by a long incubation period, by its resistance to chemicals, et cetera.

To me, it is just a plain infectious agent and should be studied in that manner.

Dr. Stamp:

I am interested in that you have early spleen involvement just the same as Zlotnik has.

With your virus and your titrations, which are tenfold all the way through, do you not have difficulty in differentiating, since your long incubation period and your widespread clinical signs over a long period of time must be difficult to assess.

Dr. Eklund:

It is really a simple matter. In our hands and with our mice the end points are sharp. They are not difficult to obtain. There is a straight line relation between the time to death and the titers. The end points are much sharper than we find with many of our acute viral infections. In our hands titration is simple.

Dr. Stamp:

I am sorry, I thought Dr. Hadlow said there was a wide scatter. This is only when the agent is given subcutaneously?

Dr. Hadlow:

The mice used to detect virus were not inoculated subcutaneously.

Dr. Burger:

I have a question to Dr. Eklund and Dr. Hadlow which may perhaps be a little premature.

You were indicating that these animals perhaps may be incompetent. Are they incompetent only against the scrapie agent or did you test any other antigens?

Dr. Eklund:

We have a battery of about 70 arbor viruses. For every one of these, the mice produced antibodies and very splendidly. So we know that they are competent for at least 85 different viruses.

FURTHER OBSERVATIONS IN MICE FOLLOWING INOCULATION OF SCRAPIE GOAT AND SHEEP BRAIN MATERIAL

C. J. Gibbs, Jr.,[1] D. C. Gajdusek,[2] and J. A. Morris[3]
(Presented by J. A. Morris and C. J. Gibbs)

In 1963 we published a note reporting development of a chronic progressive, degenerative disease of the central nervous system in mice following inoculation of scrapie sheep brain material (1). In addition, we reported confirmation of Chandler's observation (2) on development of a scrapie-like degenerative disease of the central nervous system in mice inoculated with scrapie goat brain. We have now extended these observations to include experiments to determine whether neutralizing antibody against our mouse brain agent is detectable in sheep with scrapie and in mice with central nervous system disease following inoculation of scrapie materials. In the course of this work, Chandler's observation (3) on the resistance of the scrapie agent to boiling was confirmed. In addition, information was obtained on the relative susceptibility to scrapie-like infection in male and female mice. Finally, data were gathered concerning the relative sensitivity of mice to injection of scrapie-like infected mouse brain material by intracerebral and intraperitoneal routes.

At the outset it must be emphasized that we have not demonstrated that the etiology of the illness developing in mice several months after inoculation of scrapie sheep or scrapie goat brain or mouse brain in a passage series originating with either scrapie sheep or goat brain material is the scrapie agent, although the similarity of signs of illness and histologic lesions in affected mice are remarkably similar to the signs and lesions in scrapie affected sheep and goats. The definitive determination of this point must await development of a satisfactory serologic test, or, the use of sheep and goats in suitably controlled inoculation tests. It is for this reason that we have been careful to refer to the illnesses developing in mice following injection of scrapie sheep and scrapie goat materials as scrapie-like illness.

MATERIALS AND METHODS

Study material

The infectious materials employed were brain tissues obtained from one goat experimentally infected with sheep scrapie virus and from two sheep with naturally acquired scrapie. The goat brain material was sent to us by Mr. Pattison, Agricultural Research Council, Compton. The Compton goat brain had been shown to contain the scrapie agent in tests in both goats and mice and represented the 9th passage in goats of the infectious agent since its recovery from the brain of a sheep. The sheep brains were obtained from a moribund Cheviot ram and a sick Suffolk ewe from flocks in Illinois. On the basis of histopathologic findings, illnesses in both sheep were diagnosed as scrapie.

Goat and sheep brains were shipped to us as frozen whole tissues. These were suspended in sufficient physiological saline to make a 10 percent suspension and following low speed centrifugation were inoculated into mice intracerebrally. Some of the mice, $2\frac{1}{2}$ to 17 months later, developed a degenerative disorder of

[1] Head, Laboratory of Slow, Latent, and, Temperate Virus Infections;
[2] Director of Studies on Child Growth and Development, and, Disease Patterns in Primitive Cultures, National Institute of Neurological Diseases and Blindness; and, [3] Chief, Respiratory Virus Disease Section, Division of Biological Standards, National Institutes of Health, United States Department of Health, Education and Welfare, Bethesda, Maryland.

the central nervous system associated with clinical signs of illness similar to those described by Chandler (2). Brains of the affected mice in the goat brain and sheep brain passage series were harvested and used either immediately after harvest or after storage at -60°C for several months. Suspensions prepared from the affected goat, sheep and mouse brains constituted the stock virus seeds for use in the current work.

The sera employed in serological tests were obtained from sheep and mice moribund with naturally acquired or experimentally induced scrapie or scrapie-like disease and from normal sheep and mice with histories of no known contact with scrapie affected animals.

Serologic Tests

The neutralization tests were performed with dilutions of mouse brain infected with the scrapie-like agent and inactivated (56°C for 30 minutes), undiluted serum. The serum-virus mixtures were incubated at 37°C for 1 hour and then inoculated into 6-8 gram mice by the intracerebral route. Complement fixation tests were performed in the standard way using antigens prepared from the brains from moribund and normal mice and sheep. The antigens consisted of the supernatant fluids of 10 percent brain tissue suspensions after centrifugation at 13,000 rpm for 30 minutes.

Fluorescent antibody tests were performed using the direct and indirect techniques. Brains of moribund sheep and mice served as sources of antigens. In the indirect tests used was made of anti-sheep and anti-mouse sera prepared in rabbits and conjugated with isothiocyanate while in the direct test the serum of moribund mice tagged with isothiocyanate was employed.

RESULTS

Findings in Serological Tests

The findings in the neutralization tests with the agent of the scrapie-like mouse illness and sera obtained from scrapie moribund and normal sheep and mice are shown in Table 1. It is seen that with the technique employed, no differences in neutralizing capacity between sera obtained from normal and affected animals was demonstrated. Negative results were also obtained in complement fixation and in direct and indirect fluorescent antibody tests with the same reagents.

Failure to Demonstrate Immune Tolerance in Test Mice.

An experiment was carried out in an attempt to determine whether or not immune tolerance could be demonstrated in mice injected early in life with mouse brain infected with scrapie agent derived from goat and sheep brains. This approach was taken because it was shown by Hotchin (4) that neonatal inoculation of mice with lymphocytic choriomeningitis virus induces a persistent infection in which high virus titers persist for many months in the absence of clinical signs of disease.

The results of the experiment, given in Table 2, show that no such tolerance was

Table 1.--Failure to demonstrate neutralizing antibody to mouse "scrapie" in tests performed in mice with sera obtained from sheep and mice moribund with naturally acquired or experimentally induced scrapie

Virus dilution	Scrapie virus mixed with[1]			
	Sheep sera		Mouse sera	
	Normal	Scrapie	Normal	Scrapie
10^{-1}....	6/6[2]	8/8	10/10	8/8
$^{-2}$....	8/8	8/8	10/10	8/8
$^{-3}$....	7/7	8/8	10/10	8/8
$^{-4}$....	8/8	8/8	3/3	4/4
$^{-5}$....	8/8	7/8	7/8	6/7
$^{-6}$....	2/9	0/5	0/9	0/7
$^{-7}$....	0/9	0/8	0/10	/5
$^{-8}$....	0/9	0/8	0/10	--
LD_{50}....	$10^{-5.6}$	$10^{-5.4}$	$10^{-5.4}$	$10^{-5.4}$

[1] Mixture of undiluted serum (inactivated 56°C/30 min.) and 10-fold dilutions of mouse brain infected with scrapie virus incubated at room temp. for 1 hour, then inoculated into 6-8 gm mice by IC route.

[2] Number mice developing "scrapie"/number mice inoculated.

Table 2.--Mortality and mean incubation periods in mice of different ages following intracerebral injection of 10^4 mouse $ICLD_{50}$ of "scrapie" agent

Age of mice at time of inoculation (days)	Number mice in group	Number mice developing fatal "scrapie"	Percent mortality	Mean incubation period (days)
1/2.....	46	44	96	148
1.....	26	25	96	150
7.....	39	39	100	150
14.....	31	28	91	150
28.....	18	18	100	149

demonstrated in mice injected with the agent of scrapie-like illness. Mice injected 12 and 72 hours after birth were as susceptible to fatal scrapie-like infections as were older mice injected with aliquots of the same material. Further, the age of the mice at the time of inoculation did not affect the mean incubation period of the scrapie-like illness. Thus, we were unable to obtain information which would lend support to the hypothesis that the prolonged incubation period that characterizes the scrapie-like illness in mice might have as its cause an insult to a persistent tolerant state.

Transmissibility of Clinical Signs Which Occurred in Mice Following Injection of Scrapie Sheep Brain

A study was made of the transmissibility of the scrapie-like clinical syndrome occurring in mice following the injection of scrapie sheep brain. Of 10 sheep brains used in the course of our studies as sources of inoculum for experiments in mice (Swiss-Webster strain), injection of only two of these has led to the appearance of scrapie-like signs; we have succeeded in serially passing only one of these two. The passage history of this line is shown in figure 1.

It is seen that three-fourths of the 100 mice injected in October, 1962, with a suspension of sheep brain developed scrapie-like signs in the ensuing 15 months. The first of the originally inoculated mice was noticed to exhibit scrapie-like signs 10 months after injection, or in August, 1963. Passage of the brain of one of the affected mice to 100 other mice was followed 6 months later (in January, 1964) by a syndrome in about one-half of the test animals. It is of importance to note that the original and passage materials were free of detectable PPLO, Listeria monocytogenes, Tyzzer's agent and of bacteria capable of growth on blood agar and thioglycollate.

Histopathological study of tissues of mice in this sheep passage series were carried out in collaboration with Dr. Elizabeth Beck who demonstrated that the lesions in the brains and spinal cords of the affected mice were similar to those described by Pattison and Smith as being typical of scrapie (5).

Physical and Biological Properties of Goat Passed Agent

In the study of the physical and biological properties of the goat passed scrapie-like agent which has been adapted to growth in NIH mice (Swiss-Webster strain), we have confirmed Chandler's observation on the resistance of the goat passed scrapie agent to exposure for 15 minutes to 100°C (virus suspension in sealed ampoules immersed in boiling water). The findings in this experiment shown in table 3 indicate that while approximately 4 logs of virus was inactivated by exposure to boiling water, the activity of some of the infectious particles withstood the treatment. No measurable difference in drop in infectious titer was detected between infectious material which was diluted before or after heating.

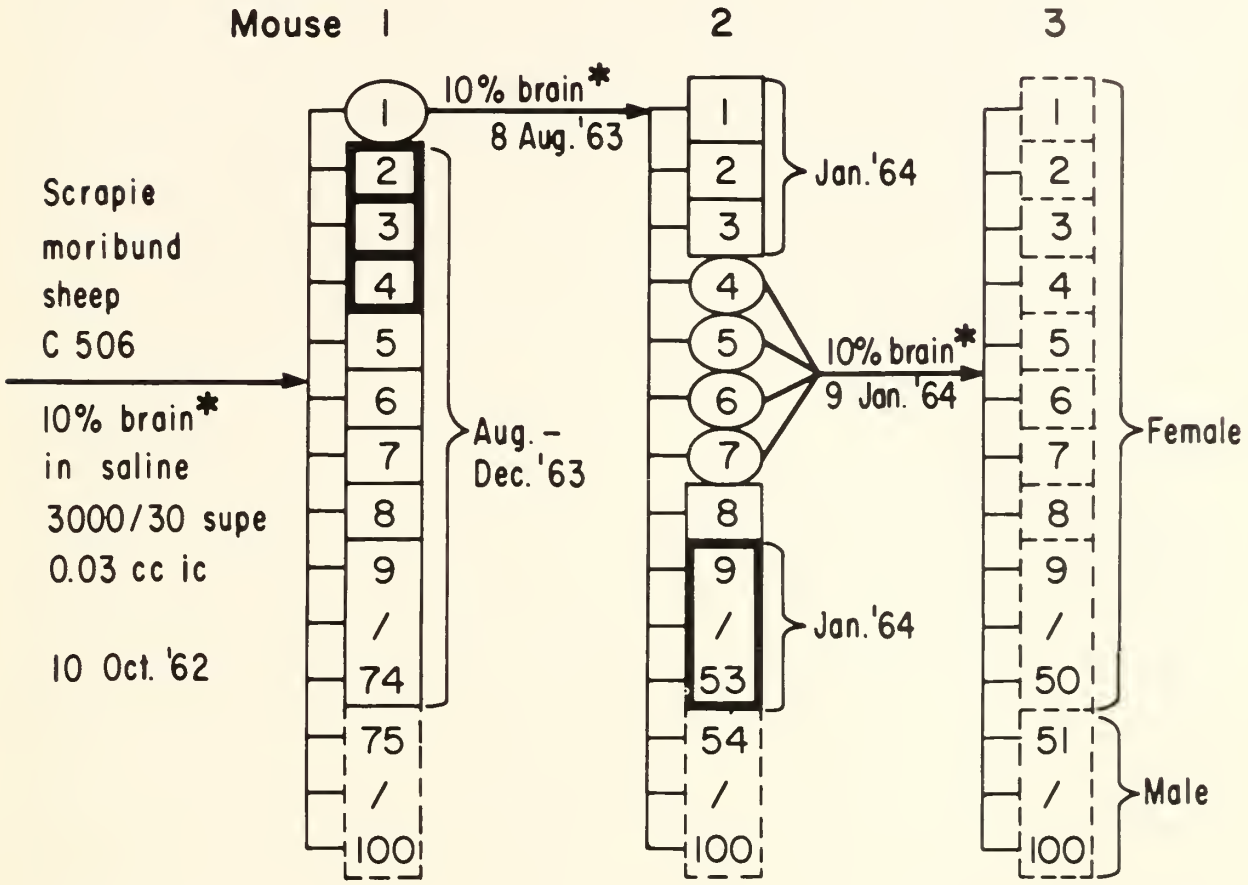
In table 4 it is seen that there was no significant difference in the susceptibility to infection between male and female mice. However, as shown in table 5 the route employed in the inoculation of mice in infectivity titration experiments was of importance. The infectious titer of goat passed scrapie virus on primary inoculation of mice by the intracerebral route was appreciably higher than in mice injected with the same material by the intraperitoneal route.

Table 3.--Resistance of mouse "scrapie" agent to exposure for 15 minutes to 100°C (boiling water)

Dilution of infected mouse brain suspension	None	Treatment	
		100°C/15 minutes	
		Dilute before exposure	Diluted after exposure[1]
10^{-1}.....	10/10[2]	5/8	4/5
$^{-2}$.....	10/10	3/6	1/5
$^{-3}$.....	10/10	0/7	1/8
$^{-4}$.....	3/3	0/10	1/6
$^{-5}$.....	7/8	0/5	0/6
$^{-6}$.....	0/9	0/8	0/6
$^{-7}$.....	0/10	0/8	0/8
$^{-8}$.....	0/10	0/8	0/8
LD_{50}.....	$10^{-5.4}$	$10^{-1.6}$	$10^{-1.6}$

[1] Exposed as 10^{-1} dilution.
[2] Number mice developing "scrapie"/number mice inoculated (post-inoculation day 712).

PASSAGE SCHEME IN 6-8 GM MICE OF BRAIN MATERIAL ORIGINATING WITH SCRAPIE SHEEP C506


Mouse 1
2
3
Scrapie moribund sheep C 506
10% brain* in saline 3000/30 supe 0.03 cc ic
10 Oct. '62
10% brain* 8 Aug. '63
Aug. – Dec. '63
Jan. '64
10% brain* 9 Jan. '64
Jan. '64
Female
Male
Mouse with "scrapie" signs, brain used for passage
Mouse with "scrapie" signs, allowed to die
Mouse with "scrapie" signs, killed for study
No "scrapie" signs, as of Jan. '64

*Contained no detectable PPLO, L. monocytogenes, Tyzzer's agent or organisms capable of growth on blood agar or thioglycollate

Figure 1.

Table 4.--Results of inoculation of goat passed scrapie agent into male and female mice

Dilution of infected mouse brain suspension[1]	Number of mice developing scrapie[2]	
	Number mice inoculated	
	Male	Female
10^{-1}.....	10/10	9/9
-2.....	9/9	10/10
-3.....	3/3	7/7
-4.....	1/1	3/3
-5.....	--	2/2
-6.....	2/2	2/2
-7.....	0/6	0/6
-8.....	2/2	0/6
LD_{50}.....	$10^{-6.7}$	$10^{-6.5}$

[1] Inoculated into 6-8 gm Swiss-Webster mice IC route.
[2] On post inoculation day 712.

Table 5.--Relative susceptibility of mice to goat passed scrapie virus inoculated by different routes

Dilution of infected mouse brain suspension	Route of inoculation into 6-8 gm mice	
	Intracerebral	Intraporitoneal
10^{-1}.....	10/10[1]	5/6
-2.....	10/10	5/5
-3.....	10/10	3/10
-4.....	3/3	2/9
-5.....	7/8	0/10
-6.....	0/9	0/10
-7.....	0/9	0/10
-8.....	0/9	0/10
LD_{50}.....	$10^{-5.4}$	$10^{-2.8}$

[1] Number mice developing "scrapie"/number mice inoculated (Swiss-Webster strain)

DISCUSSION AND SUMMARY

We have failed to demonstrate antibody directed against either the scrapie agent present in sheep brain or the scrapie-like agent present in mouse brain. There are several possibilities that might be offered in explanation of these negative findings. First, the scrapie and scrapie-like antigens were present in the test materials in amounts below detectable levels. Second, the test sera were devoid of antibody detectable by the four techniques employed (virus neutralization, complement fixation, direct and indirect fluorescent antibody tests). Third, it might be that the agent in mice is not the scrapie agent, but some other agent. However, this third hypothesis hardly fits the failure to detect specific scrapie antigen and antibody in tests employing the sheep brain-sheep serum system. Thus, the reagents or techniques employed to detect antigens and antibodies may not have been satisfactory. We are trying now to prepare adequate antigens and antisera.

Tissues and sera of sheep and mice have now been obtained from animals in various stages of disease following naturally acquired and experimentally induced infections. We plan to examine these materials in serologic tests employing a variety of techniques for the presence of scrapie specific antigens and antibodies, altering the procedures of the neutralization test, particularly the time and temperature of preincubation of virus-serum mixtures. Furthermore, since we must entertain the possibility that affected animals may not possess specific antiviral antibodies; in such a slow infection as scrapie we are also using hyperimmune sera prepared in rabbits and guinea pigs by serial inoculation of virus containing suspensions of mouse and sheep brain containing high concentrations of infectious particles as determined in infectivity titrations performed in mice. We are also using in this work ultracentrifugally purified preparations of virus.

We have succeeded in 1) serial passing in mice the scrapie-like clinical syndrome obtained in mice inoculated directly with scrapie sheep brain; 2) confirming Chandler's observation on the resistance of the scrapie agent to boiling; 3) determining the relative susceptibility of male and female mice to goat passed scrapie agent; and, 4) determining the relative sensitivity to infection of mice inoculated by the intracerebral and intraperitoneal routes. It is emphasized that we have not proven that the illness developing in mice following intracerebral exposure to bacteria-free scrapie sheep and scrapie goat brain is the etiologic agent of scrapie, although the signs of illness and the histopathological lesions in affected mice are strikingly similar to the signs exhibited and the lesions found in scrapie affected sheep and goats. The problem of identification of the mouse pathogen will be easily resolved when satisfactory specific serological tests for scrapie are developed. Our efforts are now centering in this direction.

REFERENCES

1. Morris, J. A. and Gajdusek, D.C. 1963. Encephalopathy in mice following inoculation of scrapie sheep brain. Nature 197: 1084-1086.
2. Chandler, R. L. 1961. Encephalopathy in mice produced by inoculation with scrapie brain material. The Lancet 1: 1378-1379.
3. Chandler, R. L. 1963. Experimental scrapie in the mouse. Research in Veterinary Science 4: 276-285.
4. Hotchin, J. 1962. The biology of lymphocytic choriomeningitis infection: virus induced immune disease. Cold Spring Harbor Symp. on Quant. Biol. 27: 479-499.
5. Pattison, I. H. and Smith, K. 1963. Histological observations in experimental scrapie in the mouse. Research in Veterinary Science 4: 269-275.

DISCUSSION

Dr. Eklund:

If I remember your slide on heat activation, you had a drop in titer of about three logs of virus when exposed to heat.

Dr. Gibbs:

Yes.

Dr. Eklund:

That means roughly 9,999 particles disappeared.

Dr. Gibbs:

Right.

Dr. Eklund:

And most of the particles are heat susceptible.

Dr. Gibbs:

Most are heat susceptible, but there are some that are heat resistant.

Dr. Hotchins:

In your titration, where you used the IP route, and there were survivors from a dose of virus that was lethal intracerebrally, have you rechallenged or challenged those mice or similar ones with an intracerebral shock or higher IP shock later, to see whether they were immune as a result of having gotten rid of their inoculum?

Dr. Gibbs:

I can answer that right away. The answer to the specific question is, no, we have not challenged them. We have at the present time only bled them. We have them on continuation of hold. We intend to challenge these animals intracerebrally.

Dr. Morris

Some of these mice have been on test for 712 days. If the incubation period is appreciably beyond this time then the mice will die of old age.

Dr. Gibbs

We have suitable controls that have been on equally as long.

Dr. Hotchins

If you give the IP dose and then challenge within a reasonable time, maybe two months, are they immune?

Secondly, is serum from such animals more likely to have antibodies than serum from other mice?

Finally, with some strains of LCM, to pursue this analogy further, we have what we call the high dose inhibitive phenomenon which means in a straight orderly titration where you are looking for the immediate lethal effect of LCM, very few mice die at 10^{-1}, 10^{-2}, or 10^{-3}, In some cases none. Then you get a period of death from the acute illness that comes on in 10 days or so. Beyond the end point there will sometimes be some immune mice which never show anything.

In terms of late onset disease and immunological tolerance, those mice with that history are tolerant and they succumb, at least they will show in a great number of portions this late onset of disease some ten months later.

If we postulate that your agent could not cause any immediate disease at all--if yours

is like LCM, more harmless--would you probably have to give a fairly big dose to cause an effect, and obviously if this analogy is true your virus is more capable of producing immunological tolerance. But beyond the end point there should still be mice, theoretically, which are getting too low a dose; but might be immune. You could test this theory by the experiment you suggested.

Dr. Gibbs:
Thank you.

Dr. Stamp:
I can't remember from your article in Nature, what breed of sheep were they that you used?

Dr. Gibbs:
Dr. Morris' article was in Nature.

Mr. Parry:
The black faced ones.

Dr. Stamp:
I thought it was Cheviot.

Dr. Morris:
The first sheep was obtained from Illinois. The breed was Cheviot.

Dr. Stamp:
I am interested in the Cheviot. You definitely got a positive take from the Cheviot?

Dr. Morris:
Yes but this was not transmitted.

Dr. Stamp:
From the pathology you described in Nature, I had doubts whether this was scrapie. You are not confident that you have transmitted it? They came down in about $2\frac{1}{2}$ months which in our experience with Cheviot sheep material has not been observed.

Dr. Goode:
Dr. Gordon, does that cover the question or comment you had to make? Or would you care to comment?

Dr. Gordon:
Dr. Chandler, from my staff, told me after his visit to Dr. Morris at Bethesda that the mice inoculated with Cheviot scrapie brain showed symptoms of itch. Has anything more developed from this?

Dr. Morris:
These mice relative to their scratching to us looked like minature sheep. Mice continued to show this sign.

Dr. Gordon:
This is the strain that would not pass?

Dr. Morris:
Would not pass.

Dr. Goode:
We have never had an opportunity in this country to hear on a single program discussions of so many different views on the various aspects of the scrapie problem, and the observations and interpretations upon which these views are based. I think this seminar has certainly been unique in that respect.

I also feel that we brought a lot of new information to our people particularly that we wouldn't have been able to obtain otherwise. For this we are certainly grateful.

It is gratifying also to know of the many aspects of this very complex disease situation which are not in dispute amongst the group. I feel we will always be indebted to these scientists who have come from Europe and those in this Country who have participated in the program and have made it what I think is such a terrific success.

A REVIEW OF SCRAPIE PROGRAM IN THE UNITED STATES

R. J. Anderson

We certainly are glad to have so many of you returning this morning after 3 days of very enlightened discussion on scrapie.

Before we move into this morning's program, I would like to make a few comments about today and tomorrow.

The United States Department of Agriculture is conducting a review of scrapie in the United States and the cooperative State-Federal Scrapie Eradication Program. In connection with this review, the Department sponsored the Scrapie Seminar that was held here during the past three days. It brought together, we believe, the outstanding scientists in the world knowledgeable in scrapie and scrapielike diseases. It provided a medium for reporting of research findings and expression of views and opinion on scrapie which will be most helpful to us in connection with this review.

The Department also has brought together a group of consultants to serve as evaluators. This particular group represents various segments of the sheep industry, schools of veterinary medicine, State departments of agriculture, research workers, the farm press, and regulatory officials. The Department will ask these gentlemen to assist it in its review of the program.

The first part of the meeting today will provide an opportunity to receive the views and comments that may be useful to the group in conducting this review. We are taking advantage of the presence of our British and Canadian friends and research workers to obtain the benefit of their experience and views on how best to deal with this disease.

I would like to mention just a little about how we got into a scrapie eradication program. Scrapie was first reported in this country in 1947. When two outbreaks of the disease were reported in California in 1952, the Department was requested by the sheep industry and the United States Livestock Sanitary Association to participate in an eradication program. After a thorough review of all the information known about scrapie and its occurrence in the United States, the Department decided to join the States in a cooperative effort to eradicate the disease from this country. This decision was based on the following Department philosophy:

Every means available should be used to prevent the introduction of foreign diseases into this country. If introduction or an outbreak occurs every effort should be made to eradicate the disease where knowledge and tools are available. Where such tools and knowledge are not available, then we believe every means should be used to control and contain it until research provides us with the information needed to achieve this objective.

A review of the facts available in 1952 showed that:

First, scrapie was new in the United States.

Second, it was not widespread, and we believed that it was largely confined to a few flocks.

Third, that although more knowledge was desirable, we believed that sufficient information was known about the disease to warrant adopting a program of eradication, because to delay action until more was known about it would result in its becoming firmly established in our sheep flocks and would prevent or seriously handicap eventual eradication of the disease.

Since then the Department has made a continuing review of the program in light of research information developed and experience gained and has made changes as warranted.

That is the main purpose of this meeting today, to review the most recent research and knowledge about the disease and the experience that has been gained during the past 10 or 12 years in this country in dealing with it, and to determine whether or not we are following the right course or to determine whether further changes should be made in the program.

I would at this time like to introduce to you the group of consultants that are working with us in this endeavor. I will take them alphabetically.

First is Dr. W. L. Bendix, State Veterinarian of the State of Virginia, Richmond, Virginia.

Mr. Roy A. Gilman, a breeder of sheep and Executive Secretary of the Hampshire Breed Association, Stuart, Iowa.

Dr. W. J. Hadlow, research scientist of the United States Public Health Service, Rocky Mountain Laboratory, Hamilton, Montana.

Mr. Sherwood Jackson, a vocational agricultural instructor and a Suffolk breeder from Seneca, Illinois.

Dr. Rue Jensen, Dean of the College of Veterinary Medicine, Colorado State University, Fort Collins, Colorado.

Mr. T. A. Kincaid, a breeder of both purebred and commercial sheep from La Vernia, Texas, representing the National Wool Growers Association.

Dr. L. A. Rosner, State Veterinarian of Missouri and President of the United States Livestock Sanitary Association, Jefferson City, Missouri.

Dr. James E. Stuart, who was one of the first regulatory officials in this country to have experience with scrapie, recently retired and former Chief of the Division of Animal Industry, State Department of Agriculture of California, Sacramento, California.

Mr. Paul W. Swisher, a sheep breeder and Commissioner of Agriculture, State of Colorado, Denver, Colorado. Commissioner Swisher is also a member of the National Association of State Departments of Agriculture.

Mr. Olin H. Timm, a sheep breeder, Dixon, California, representing the California Wool Growers Association and an industry representative, U.S. Livestock Sanitary Association.

Mr Ralph S. Yohe, a sheep breeder from Racine, Wisconsin, also a former official of the American Rambouillet Breed Association and who is also associated with the Wisconsin Agriculturist, which is one of the leading farm magazines in Wisconsin.

Paper No. 39

CERTAIN BIOLOGICAL SITUATIONS IN THE ANIMAL AND PLANT KINGDOMS POSSIBLY RELEVANT TO SCRAPIE

H. B. Parry[1]

The concept of a transmissible particle or provirus being intimately concerned with a recessive gene in the causation of scrapie has proved difficult to accept, especially by those of us nurtured in the bacteriological era of veterinary science. It involves a new appraisal of some of our traditional beliefs, a re-appraisal which would perhaps be less difficult if it could be shown that the kind of phenomenon described in Paper 16 was not peculiar to scrapie, but was recognized in other biological systems.

Figure 1 provides a short summary of some possibly analogous situations in other mammals, insects and plants. To these may be added Aleutian disease of mink and leucosis of cattle; avian leucosis and Marek's disease are probably not closely similar.

Scrapie, however, shows a number of clinical, pathological and epidemiological features which are akin to a wide group of neuromuscular degenerations in man (Parry, 1962; Beck, Daniel and Parry, 1964). A summary is shown in table 1. Many of these are familial

[1] Nuffield Institute for Medical Research, University of Oxford, Oxford, England.

Analogous situations in lower animals and plants

Lesion	Host	Genetics	Agent	Spread
Leukaemia	mouse	certain strains	virus	non-infectious thro' mother
Mammary carcinoma	mouse	certain genotypes	bittner milk factor	non-infectious thro' mother's milk
CO_2 sensitivity	drosophila	complex	hereditary virus	mainly non-infectious
Caterpillar death	abraxas grossulariata	lacticolor variety	polyhedral virus	in recessive genotype only
Tumours	plants infected with phytomomas tumefaciens		infective particle	non-infectious
Perithecial formation	fungus aspergillus	nuclear gene	self replicating cytoplasmic particle	thro' hereditary mechanism

Figure 1.

Table 1.--Some syndromes of familial neuromuscular degeneration in man with affinities to scrapie

Syndrome	Author	Location	Age of onset	Possible Inheritance
Muscular dystrophy (Progressive)	Minkowski & Sidler, 1928.	Switzerland	15-30	Rec. x 2
Myopathy	Barnes, 1932.	England	35-50	Dom.
Muscular dystrophy (limb-girdle)	Stevenson et. al. 1954-55	Ireland	15-40	Rec.
Hereditary ataxias	Schut et al. 1951, 1954	Holland and U.S.A.	17-35 (26.5)	Dom. + sex-link
Amyotrophic lateral sclerosis	Kurland & Mulder, 1954-5.	Guam	25-55 (44)	? Dom.
Kuru	Zigas & Gajdusek 1957; Bennett et. al. 1959.	New Guinea	5-60 (5-15) (25-45)	? Dom.
Parkinsonism-dementia	Hirano, Kurland et al. 1961	Guam	32-64 (54)	

and are thought to be heriditary. Until very recently there was no evidence for the presence of a transmissible agent in any of them. However Zil'ber and his colleagues (Zil'ber, 1962) have recently demonstrated in a human case of amyotrophic lateral sclerosis a virus-like particle causing on inoculation into monkeys the specific anterior horn cell damage of A.L.S. He considers that the situation in A.L.S. may be closely similar to that we have postulated in scrapie, since Mulder and Kurland (1955) have good grounds for suspecting A.L.S. on guam to be genetically determined.

Evidence is thus coming to hand that the gene-provirus relationship postulated in scrapie is not a phenomenon unique in the biological world or in mammals, but may represent a type situation for these chronic neuromuscular degenerations of man, for which so little can be done.

DISCUSSION

Dr. Dickinson:

This agent is contact transmissible at birth.

Mr. Parry:

Yes, this may be true. I only wanted to draw attention to the very broad outline.

Dr. Dickinson:

I must apologize for my interruption earlier. It was very rude, but I am sick of listening to Mr. Parry on these issues. He does not read the literature. He has read it a little more since his original paper, but I want to take him to task on one or two points.

Do you know of cases of transmission, the very suggestive cases, to make it likely that the mouse mammary tumor agent is transmitted by the male? Do you know of these? I am going to press these points. I have read the literature very extensively. Bittner, 1950, has one very suggestive case. Muhlbock,

1952. And I can give you the reference. Also Foulds, 1949: Three cases, independent people. It looks funny. The male sometimes does something with this agent which classically goes through the mother. I'd like to make some more points. I am not finished here.

Dr. Anderson:

Pardon me just a moment. Mr. Parry, I don't know whether for the purpose of this meeting it is relevant to argue the points that may be associated with your presentation. If your presentation was merely to present the areas in which there may be similarity, then I think we will leave it at that.

Dr. Dickinson:

I'd like not to leave it, Mr. Chairman, if I may, because some of these cases affect the important issue of whether males can sometimes be transmitters of these agents, a fact which was not mentioned a single time in this presentation. This is important. Sometimes it goes through the milk. Sometimes it is transmissible by contagion at birth; there is another fact which Parry didn't know. All I am saying is--and I want to say these models are like scrapie, but I want to say it on the basis of the full presentation of the evidence, which make it look very scrapie like, and there are very suggestive cases in some of the instances that males are involved, and that's what the table on the board over there suggests, reference to possible ram transmission of scrapie.

Mr. Parry:

Mr. Chairman, I think we are trying to do the same thing, Dr. Dickinson and I. I was not concerned to put this in evidence for my concept of scrapie as against his. I merely wanted the meeting to know that these models did exist. They can look into them for themselves.

Dr. Dickinson

No, they can't. I would not have the presumption to bring these cases before this audience if I had not done my level best to cover the literature on them.

Mr. Anderson

Well, it is obvious that this type of difference of views could be carried on for some period of time. I think for the purposes of this meeting--

Dr. Dickinson:

I would like to point out, just for the benefit of everybody here, that the classification of these so-called genetic anomalies that we have dealt with in the second slide there, this classification very often involves circular arguments of the type which has led to such disastrous results with some scrapie interpretations. I am not saying that these interpretations in these cases here are wrong. I am saying the same pitfalls exist for those cases, so don't take things as genetic until the evidence in those cases is very stong, and I hope you will back me up on this.

Mr. Parry:

I couldn't agree more with Dr. Dickinson about this point regarding the evidence that the hereditary nature of some of these human syndromes and the medical clinical difficulties that my colleagues are faced with are even greater than ours.

Dr. Hadlow:

Dr. Eklund and I have embarked on the study of scrapie merely as one of the tools for getting a better understanding of this phemonenon which is involved in these chronic viral infections, and since our base of reference is human disease we are using these largely as tools to get a better understanding, because our feeling is that the basic pathogenic mechanism involved in disease such as scrapie most likely also operates among the human population. We feel that such mechanisms aren't limited to a very narrow spectrum of the animal kingdom, and it is on this basis that we are pursuing such diseases as scrapie and Aleutian disease. The hope is that in studying these we will evolve techniques which could be applied to studying comparable diseases in man. Certainly from the few examples that Mr. Parry cited I think it is reasonable to assume that there are diseases affecting man which evolve via the same or similar basic pathogenetic mechanism.

Dr. Stamp:

I would like to make an observation, and this is that I find this sort of reference to

other diseases, especially in man, quite interesting; but I would like to point out that, first, we know a great deal more about scrapie disease of sheep than is known about any of the conditions in man that Mr. Parry has mentioned. It seems to be rather dragging a red herring in to suggest that there is knowledge about these diseases that might help scrapie. I would suggest it was much the other way around. We know a lot more about scrapie, and therefore our knowledge of scrapie may help in the investigation of these diseases. I think the inference Mr. Parry has made is that knowledge of these diseases helps us in our research into scrapie. I don't believe this for one moment. I am firmly convinced that scrapie is not the only disease of its type and if it turns out that we get the full answers to scrapie, I am quite sure there will be many other diseases of a similar nature explained; but merely to drag a textbook of pathology of the unknown diseases over the screen isn't going to help us at the moment with scrapie. We know more about scrapie disease of sheep than probably is known about any other central nervous system disease where a virus has not yet been isolated.

I am rather tired of the suggestion that we know nothing about scrapie. We know a great deal about scrapie. We know more about scrapie, for instance, than is known about allergic encephalitis. If you go through the literature about allergic encephalitis you will learn the literature is more incomplete than the literature about scrapie. Therefore, dragging this morning a textbook of pathology in front of us isn't helping one iota. Little is known about these diseases. We know something about scrapie.

Paper No. 40

THE CONTROL AND POSSIBLE ERADICATION OF NATURAL SCRAPIE IN SHEEP. I. THE DEFINITION AND DIAGNOSIS

H. B. Parry[1]

The criteria of natural scrapie in sheep, upon which our own control programme has been based are briefly as follows:

CLINICAL SIGNS

The 5 syndromes of classical scrapie given in Paper No. 8, fig. 1. We recognise that in addition within scrapie families other syndromes of neurological illness (Paper No. 8, fig. 2) occur which account for about 5 percent of all chronic neurological cases in these flocks. They are not classified as scrapie, although their occurrence is considered as highly suspicious. It is possible that these atypical syndromes may prove to be pleiotropic expressions of the scrapie gene, a phenomenon well recognised in the genetics of human diseases as leading to a diversity of clinical expression in different genotypes.

COURSE AND OUTCOME

This is almost invariably progressive and fatal. We have only had 2 out of 1500 cases show a remission. Of the 1500, some 500 have been observed until they were moribund. For practical purposes remission of the progressive course of the disease occurs in less than 1 percent of cases.

[1] Nuffield Institute for Medical Research, University of Oxford, Oxford, England.

GENERAL PATHOLOGY

This is not diagnostic and there is often little to see with the unaided eye. Changes occur in many cases in the skeletal muscles, although the massive pale foci observed in some early cases have only been found infrequently since. The endocrine glands show deviations from the usual weight patterns, which suggest some general disturbance of the target organs under control of the pituitary.

NEUROPATHOLOGY

A primary system degeneration in (i) the afferent motor pathways to the cerebellum, the olivo-ponto-cerebellar tracts, commencing at various points, e.g. in the mossy fibres of the cerebellum or the bouton terminaux of the pontine nuclei, with retrograde degeneration along the axon and subsequent vacuolation of the neurone; (ii) the hypothalamo-neurohypophysial system with primary loss of neurones in the supraoptic and paraventricular nuclei and possibly other nuclear masses in the hypothalamus, with consequential degeneration in the tract from the supraoptic nucleus to the posterior lobe of the pituitary and disturbances in the distribution of neurosecretory material.

There are other changes in the brain, which are probably essentially non-specific.

THE PRESENCE OF A TRANSMISSIBLE SCRAPIE AGENT

LABORATORY TESTS

All attempts to find a laboratory test which might be applied to blood have failed. There is no obvious correlation between sheep with or subsequently developing scrapie and the potassium red cell type, haemoglobins A, AB and B, blood groups R, O, i and X, lipoproteins, serum aldolase, or other chemical and cellular constituents more commonly investigated.

FLOCK AND FAMILY HEALTH RECORDS

In cases where adequate records are available, these are proving a most useful indicator of the probable cause, and wherever possible are taken into account.

RECOGNITION OF SCRAPIE GENOTYPES

The 3 genotypes, ss, sS and SS, called for simplicity black, grey and white (see Paper No. 7), are being assessed on the basis of the occurrence of scrapie among parents, progeny and close relatives. In addition a programme to mate rams of uncertain genotype with grey ewes and observe the incidence of scrapie among their progeny is in hand.

DISCUSSION

Mr. Parry:

Now, the last point I would like to make is that I think we are agreed that there is a hereditary component, genic. It may be a lethal gene, or, in view of what has been presented this week, in addition a susceptibility resistance gene. These are not necessarily the same. The first one is subjected to considerable selection pressure, owing to the fact that these animals with this lethal gene are preferred by sheepmen. I don't want to say more, I repeat that--my sheepmen.

With regard to susceptibility and resistance, presumably this is due to some pre-existing substrate mechanism in which there is no selection pressure one way or the other, so it is possible that we have both situations. I am not saying which is right. I want you to consider whether both may be present.

And there is the question of the cytoplasmic hereditary component through the maternal transmission, if this is established, which we have heard about.

Mr. Parry:

I wanted to summarize our position, because this is the basis on which I and my flockmaster group are approaching the practical attempt to contain scrapie. This is why I wanted to make myself quite clear, because I thought perhaps I was being challenged before as to what my diagnostic criteria were.

We take the view that in the field, as we can see--and I will draw your attention to the data that I showed you on Tuesday on the introduction of rams into clear flocks, of white Suffolk ewes into an infected environment, and the table shown by Mr. Draper of this one ram from which no progeny had become affected in a highly infected environment--which we will call the Chinese white--these lead us to the view that in the field in Britain, in our small breeding population, we have as yet failed to detect any transmission by contagion.

Let me make it quite clear this doesn't mean that it does not arise. It is possible that the artificial disease is creating a new situation, and from what I have heard I suspect in the next 6 months my own experimental breeding trials will go haywire, because I have a double situation of an agent which has

been transmitted artificially, as well as the natural genetic or genic situation; that is, that in Britain there are three centers which are heavily contaminated with both artificial and natural scrapie--Compton, Moredun, and my own premises. I am not certain that we have yet gotten any contamination of my flocks in the field with the artificial agent. It may be that I shall have to be very much more careful about visiting, and so on. So I would say that this is the basis on which we have hitherto attempted to contain scrapie, and I think we shall continue to do so.

Dr. Gordon:

I take exception to one thing you have said, Mr. Parry, and that is that there are contaminated areas in England and Compton is one of them. There are certain places in Compton that I can assure you are not contaminated with scrapie, because many susceptible sheep have been bred there for years and scrapie does not occur in the clean area of Compton. It has of course been produced in other parts, and the places that are contaminated are the places where we test sheep for susceptibility. So, please in this record don't say that the whole of Compton is contaminated.

Mr. Parry:

My apologies, Dr. Gordon. I accept that. I was talking in broad terms, as Dr. Dickinson was yesterday about Edinburgh.

Dr. Gordon:

I want this to be quite clear, because it is important in assessing all our sheep experiments.

Dr. Stamp:

Mr. Parry this morning has merely reiterated what we have been arguing about for 3 days. He suggested that the muscle lesions are important this morning. If they are, why did he not present the facts and the evidence 3 days ago? He was challenged on this, challenged for a whole morning by me on this subject, and he never presented any facts whatsoever to support his idea that muscle was involved in scrapie, except to say that Professor Daniel is publishing an article in Brain, and he didn't even say what was being published.

I would deny wholeheartedly that muscle lesions have anything whatsoever to do with scrapie, and if you are dealing with the naked eye lesions that Mr. Parry mentioned this morning, we are in complete opposition to this idea. Mr. Parry has to show some better evidence than he has shown up to the moment. He published an article in the Lancet--he published an article in The Veterinary Record, showing lesions in muscles which, as I said on Monday, anyone could see, never mind a pathologist. That article has never been retracted. These statements stand as evidence of scrapie in Parry's view, and until such time as he writes an article saying that these lesions do not represent scrapie, then I must assume that such lesions are part of the scrapie pathology according to Mr. Parry. If this is so, then I flatly contradict it and say we do not think so. This is our position.

The second point is that Mr. Parry again drew a red herring over the pathology. He gave the pathology as he saw it. I gave the pathology as I saw it over the last few days. We have debated it and argued it. Why should we go back to the arguments this morning? I flatly contradict that in Cheviot scrapie--and Cheviots take scrapie as well as sheep of other breeds--the lesions that Mr. Parry has mentioned this morning do not exist, any more than the muscle lesions do. Therefore, if scrapie is as Mr. Parry sees it and is based on the pathology that he has described to us this morning, it appears to me to be an entirely different disease than the one that I know as scrapie. We are talking about two entirely different diseases.

Thirdly, Mr. Parry has dragged a lot of genetic red herrings across the picture this morning about which again we have been arguing for 3 days. All that Mr. Parry has done this morning is emphasize his side of the story, and this is to what I am objecting.

I would say that if Mr. Parry is giving his story again this morning then Dr. Dickinson should get up and give his story again. This is the unfairness of this. We are here this morning as an intelligent group of men to assess the findings of the last 3 days, not to hear Parry's views again.

Mr. Parry:

The point is clear what the criteria were, Mr. Chairman, that we in our breeder group accept for scrapie. It may not apply to Cheviots. I know nothing about Cheviots. Please, it only applies to the breeding population with which I have knowledge, but these are the things that we accept and on which we base our whole experimental program to attempt to contain the disease. If they are not acceptable and not applicable to other breeder populations, this will have to be established; I don't know. I thought I made it clear that this applied only to the breeding populations with which I am familiar.

Dr. Stamp:

If this is so then I will talk about Suffolks. I want to know if the evidence that you have just given about muscle lesions is confined or mainly confined to Suffolks.

Mr. Parry:

No, it is not.

Dr. Stamp:

Tell us the breeds with which you are concerned.

Mr. Parry:

It is also in Hampshires.

Dr. Stamp:

Which breeds are in your eradication methods that you are going to talk about this morning?

Mr. Parry:

Both of them.

Dr. Stamp:

The Hampshire and the Suffolk, these are the two breeds. I talked about Cheviots because I know about Cheviots, but I know equally well about Suffolks, and I deny categorically that muscle lesions occur in scrapie in Suffolks in Scotland. I also deny that the English sheep which come up to Scotland that take scrapie have muscle lesions and when I say muscle lesions I am not talking about the microscopic lesions, I am talking about the macroscopic lesions Mr. Parry has mentioned this morning.

Now may I ask Mr. Parry a question. If you find muscle lesions such as you describe, and you do not find vacuoles and all the lesions that I quoted, do you diagnose this as scrapie?

Mr. Parry:

No, we do not. We take the whole picture.

Mr. Stamp:

I am not asking whether you take the whole picture. In scrapie you must have some criteria on which you pin your diagnosis. If Mr. Parry says muscle lesions are part of scrapie, then muscle lesions must always be present in scrapie. Are they?

Mr. Parry:

Dr. Stamp, I think we are at cross purposes.

Dr. Stamp:

We are not at cross purposes. We were at cross purposes earlier in the week but then you accepted that neuronal vacuolation was your method of diagnosis. Is it or is it not?

Mr. Parry:

I stated what our method of diagnosis is. Now, with regard to muscle lesions, if you read the paper it is only in a small proportion of cases in which we get these massive macroscopic lesions.

Dr. Stamp:

This was not so in your paper in The Lancet.

Mr. Parry:

I know it was not. We had a series of 45 in which we found it. In the International Congress of Neuropathology in Munich you will find it referred to, and it was stated more fully in a communication given to that Congress.

Mr. Parry:

I know. Dr. Zlotnik was at this Congress, and you did not withdraw the fact that muscles were involved in scrapie.

Mr. Parry:

No. We have no reason to.

Dr. Stamp:

You still by inference suggest that these naked eye lesions that you described in your first article are part of the scrapie story. Are they or are they not?

Mr. Parry:

We said at Munich that in the series we examined we had found these gross lesions extremely uncommon.

Dr. Stamp:

If this is so, may we forget the gross lesions this morning? This is what I am asking. Because Mr. Parry found them once in one series and didn't find them in the next series, which of these two series is Mr. Parry accepting as being typical of scrapie? The first series that you described in the Lancet or the second series that you described in Germany?

Mr. Parry:

The second series we described in Germany. After all, we live and learn. We try to live and learn. The first series we may have had an unusual sample.

Mr. Chairman,

I am not sure whether this is absolutely relevant.

Dr. Stamp:

This is quite relevant. Mr. Parry has dragged out the fact that the naked eye muscle lesions are part of the scrapie story. He has stated this this morning, and I want to get this quite clearly clarified here. Are these obvious, naked eye muscle lesions present in a Suffolk sheep affected with scrapie and if a veterinarian goes on to the farm, does a post-mortem examination, is this veterinarian expected to find these lesions or is he not?

Mr. Parry:

No.

Dr. Stamp:

Thank you.

Dr. Gordon:

I merely wanted to interject that this polemic discussion is getting nowhere. There are two views. Parry has already presented all the evidence he can present on this, and others have been presenting opposing evidence during the week. Surely the panel can assess this evidence without having to listen to this struggle over again.

"The Control and Possible Eradication of Natural Scrapie in Sheep;" 9 a.m. Mr. Parry and Mr. W. Steele. "The Relationship of Scrapie to the Live-Stock Industry;" 9:15 a.m. Mr. W. Steele, "The Practical Problems of Flockmasters." The last two are subjects that sheep breeders here will wish to hear. It is now a quarter till ten so if we could please return to the agenda, the business of the meeting can progress, but if we go on like this we will get back to another 3-day discussion.

Dr. Anderson:

Mr. Parry, will you proceed.

Dr. Stamp:

Mr. Chairman, I am sorry. This is not on the agenda--what was given this morning. This should have been on the scientific agenda. We didn't argue this before. If in fact this is going to be a scientific session again this morning--and I understood it wasn't--then it must be a scientific meeting again and we must discuss it. We cannot have one view overemphasized one way or the other.

Dr. Anderson:

The presentation this morning, as I mentioned, will appear in the scientific section of the seminar.

Dr. Stamp:

But it shouldn't, sir. This is the point. Why should I have been pushed for time yesterday afternoon?

Dr. Anderson:

I felt that probably some of the others were pushed, too, and I want to apologize to you for not having sufficient time. If there are other points that you or anyone else would like to pursue in the research aspect later on, we can schedule a period this evening where everyone will be able to go over these issues again, but I think for the sake of our time today I would ask that you bear with me and permit us to go ahead.

Dr. Stamp:

Sir, I must register a protest for the record and say that I do not agree with that which Mr. Parry has said, and that I most certainly wish it to be known that I think it unfair that these matters should have been brought up twice in this presentation.

Dr. Dickinson:

Could I merely support wholeheartedly and in every detail the comments of Dr. Stamp, and could I further ask that in any future consideration of these data one reads them only in reference to the specific denials offered to me on Tuesday of some of the things which are now no longer denials.

III. PRESENTATIONS AND STATEMENTS RELEVANT TO THE CONTROL AND ERADICATION OF SCRAPIE, AS PRESENTED IN CONJUNCTION WITH THE SEMINAR

Paper 41

THE CONTROL AND POSSIBLE ERADICATION OF NATURAL SCRAPIE IN SHEEP. II. THE CONTROL OF DISORDERS IN WHICH HEREDITARY FACTORS ARE OF PARAMOUNT PRACTICAL IMPORTANCE: SOME RESULTS WITH SCRAPIE IN GREAT BRITAIN, 1954-63

H. B. Parry

GENERAL CONSIDERATIONS

The recognition of the possible role of hereditary factors in animal disease depends on the compilation of the right kind of data. Such data are rarely available without special recording techniques and are required over a period of years. The basic unit for studies of hereditary factors is the closed breeding population or breeder group, within which interchange of breeding animals is confined. The breeder group may be widely dispersed geographically and the accumulation of data on a local or even regional basis, which has proved so rewarding in the study and control of infectious diseases, may fail completely to reveal the presence of important hereditary factors.

The most important requirement is a technique for the compilation of accurate records over long periods by a number of breeder groups, some of which suffer from the defect under investigation and some of which do not. The special recording techniques must have the complete support of owners and stockmen, with the kind of safeguards and checks discussed in Paper 10. Without complete cooperation of the practical men with the scientist, the data can be very seriously biased without anyone being aware; especially is this true in respect of negative evidence, i.e. of absence of a defect, which can be of great importance but is so often unreliable unless supported by meticulous written records compiled under independent supervision.

The control and possible eradication of genetic defects i.e. those mediated through the chromosome, present problems essentially different from those due to infectious or contagious microbial agents. Defects due to a simple dominant gene with full penetrance or complete manifestation, which can be readily observed, are of course readily eliminated by culling affected individuals, and all their progeny. Those due to a recessive gene or to more complicated genetic situations present a much more difficult problem, and only the situation of a simple autosomal recessive gene will be considered.

With a recessive gene total eradication of the gene from a breeding population may never

[1]Nuffield Institute for Medical Research, University of Oxford, Oxford, England.

be possible. In affected breeds, our aim must be to reduce the frequency of the gene in any breeder group to such a low level that the incidence of the disease is so small that it is not of any economic importance. In unaffected breeds, a constant watch must be kept to recognise any appearance of the disease. This entails continuing vigilance of complete breeder groups, for it is quite beyond the scope of the individual flockowner, unless his operations are on the scale of a self-contained breeder group and he has scientific assistance.

A practical programme for the control and containment of a defect due to a simple recessive gene can be based on the organisation of collaborative closed breeder groups of individual owners under independent scientific supervision. An example is the Suffolk & Romney Breeder Groups of the British Sheep Society organised by the Sheep Development Association in the United Kingdom.

In such Breeder Groups there is a mutual interchange of information, which is treated as strictly confidential within the group. Every effort is made to select rams thought to be free of the gene. In the early stages of a programme such assessments have to be based on the available records, which are often insufficient for the purpose, but these assessments can be kept under continuous review and if new information comes to hand, then the breeders concerned are advised accordingly.

In the later stages of a programme, animals shown to be clear of the allele by suitable test mating procedures (see the SS ram described in paper 15, table 1) or suitable progeny are used as the foundation stock for building up strains and families free of the disease and of low gene frequency.

Any programme for the control of a gene must be carefully integrated with the long-term breeding policy of the breed, and be intimately associated with the development of the factors economically important for the future of the breed. It is of little practical value to produce a strain free of any defect which is not economically viable under contemporary agricultural conditions.

The assessment of the results of a programme is extremely difficult until such time as the defect may perhaps become virtually absent from a previously affected breeder group or from a section of an affected flock, in another section of which the defect is deliberately being propagated. Data of this nature require many years to collect and it is as yet too early to do more than report possible trends.

The basic difficulty is that one cannot arrange for satisfactory control flocks nor make proper calculations of the likely incidence in a flock had they not been collaborating in a programme. The nature of this difficulty has been discussed by Draper in Paper 16, in which he demonstrates how the gene frequency may build up in a flock with the appearance of an apparent 'epidemic' or equally well decline and lead to the 'disappearance' of the disease.

Any assessment while a programme is in progress must therefore be subjective and incomplete. In practice such assessments have to be made relative to individual flocks, since the economic future of such flocks may depend on such assessments; to date our assessments relating to scrapie have gained the full support of the flockowners and shepherds with adequate experience of this particular problem.

SOME RESULTS WITH SCRAPIE IN GREAT BRITAIN, 1954-63

An estimate of general incidence must be only a crude estimate; no statistics are available, and scrapie is not a notifiable disease. Such information as I have concerns England principally.

Scrapie is extremely uncommon in commercial sheep in the Midland and Southern counties of England at the present time. When cases have come to our notice they have been almost always in ewes of one particular white-faced cross. The half million local sheep of South-eastern England are probably completely free, and it is very rare in the breeds of the Welsh Marches, and South-western England. Most sheepmen in these areas are quite unfamiliar with scrapie, have not seen it, and until the publicity of the last few years had previously never heard of it.

Scrapie has recently been recognised among some upland breeds, and there are pockets in certain hill country, where the disease is well known.

The main incidence of scrapie would probably be found in a small group of highly improved flocks of certain breeds, mainly producing rams for crossing on commercial grade ewes for fat lamb production and in certain local areas such as East Anglia, used as commercial ewes, either pure or crossed with the white-faced first cross noted above.

Thus the incidence of scrapie is extremely variable with the great majority of the sheep flocks completely free.

An estimate might be 5000 cases p.a. among the 23 million sheep in the United Kingdom, of which some 11 million are breeding ewes, with an upper limit of 10,000 cases; say an attack-rate of 0.5 per 1000 breeding sheep. Such an attack-rate is not of any direct economic importance to the sheep industry as a whole, but scrapie can be of major concern to that small group of breeds and flocks, in which a much higher attack-rate does occur. In my recorded population of about 10,000 breeding ewes of 10 breeds about 100 cases occur p.a., but confined solely to 2 breeder groups with about 5,000 breeding ewes.

Some general results are available for recorded flocks under voluntary supervision. In these flocks no attempt has been made to immediately isolate or quarantine affected sheep or their progeny, but any animals suspected of being affected with scrapie have been culled for slaughter and as many of their progeny as possible also. Any ram progeny of an affected parent are only sold for commercial meat production and are not made available to purebred flocks. All the female progeny go for slaughter as soon as this is practicable; sometimes the need for female flock replacements is such that these female progeny are retained for a time, but none of their offspring are retained for pure breeding. Such a policy would reduce the frequency of any recessive gene if one be involved, but would not, of course, take account of heterozygous (gray) animals born of unaffected parents and relatives, nor of the progeny of potential cases of scrapie, which died before manifesting the disease. Thus it might be expected to reduce the incidence of scrapie in many flocks, but make little impact of the disease arising from unaffected carrier animals.

In describing these provisional results the following terms have been used: The attack-rate is the number of manifest cases of scrapie per 100 breeding ewes occurring in a breeding year. The incidence expressed as a similar percentage, is a less precise term used to describe the approximate manifestation over a period of years.

A flock or flock-group, e.g. group of flocks forming a breeder group, is considered to be free if over 5 years or more no manifest case has been observed in the home sheep and no case in sold sheep has come to notice. If affected, a sporadic incidence is 1 percent, a low incidence 1 - 3 percent, a medium incidence 4 - 10 percent, a high incidence 10 percent and above.

4 flock-groups, 3 pure-bred and 1 cross-bred have adequate and supervised records for 7 years or longer; one of these groups is free of scrapie; 2 of the affected flock-groups have a low to medium incidence, while in the third it is sporadic. 5 other flock-groups with good records, supervised for 5 years, but not yet semi-closed or closed breeding population, are probably free. Thus the incidence varies very much between different flock-groups, although direct and indirect contact between animals from affected and unaffected flock-groups occurs frequently at Agricultural Shows and on certain farms. It occurs in pure-bred and cross-bred sheep, of long and short-wooled breeds, on mountain and lowland.

IN AN AFFECTED FLOCK-GROUP

Of 12 free flocks, 9 have used many rams from flocks with affected animals during the period of the rams' breeding and rearing. In 9 flocks an occasional case has occurred giving a sporadic incidence, often with periods of several years without a case.

Among the initial high incidence flocks the attack-rate has declined in 3 from 8.6 percent to 1.8 percent, while in 2, in which several stud rams used extensively were subsequently affected, it has remained about 10 percent. In one flock thought to be free an attack rate of 15 percent appeared suddenly in females of certain families, but suspected cases in sold sheep had been reported for several years.

The striking feature is the wide fluctuations in the attack-rate in different flocks over a period of years and even from year to year, the disease becoming absent and then reappearing in a capricious manner, the flock meanwhile being exposed to apparently similar environmental factors. Many flocks remain free in spite of using rams born and reared in affected flocks. In some an occasional sporadic case occurs, but the disease does not spread; in others with no recorded cases for years the disease may appear suddenly as an "epidemic'.

SOME DETAILED RESULTS FROM SUPERVISED FLOCKS

Data were given in paper 12, Figures 1-6, to show that (a) Scrapie does not necessarily follow the introduction and use in a clear flock of rams subsequently affected by scrapie, either among the flock ewes, nor among the ewes in-lamb to the rams developing scrapie within 6-8 months of the mating, (b) scrapie does not necessarily occur among the daughters of an affected ram (c) the introduction of females (probably white) from a clear flock into a high incidence flock is not necessarily followed by the appearance of scrapie among the introduced sheep (d) the intermingling of affected and unaffected breeds on the same premises does not necessarily lead to the appearance of scrapie in the unaffected breed although cases are occurring in sheep of the affected breed.

In several flocks we are deliberately breeding black scrapie prone sheep in one portion of the flock, while either maintaining the rest of the flock free of scrapie or where scrapie is already present endeavouring to reduce the attack-rate in the remainder of the flock. At the bottom of paper 12, figure 4, some figures are given for one such flock, which has been used as an experimental breeding flock in which all females reared to 9 months and suitable for breeding have been retained and no breeding animals have been culled except on the grounds of old age or some serious incapacity such as a damaged udder. The flock incidence between 1955 and 1960 was some 10 - 15 percent p.a. It rose to 16 in 1961, partly due to certain deliberate experimental matings, fell to 7 percent in 1962 and is 2 percent for the first half of the current year. In this flock we hope to use the test-mated 'Chinese White' ram, (see Paper 14) and to assess his ability to throw progeny which will not develop scrapie.

The use of rams proven clear by Test-Mating is the crux of any programme for the control of a recessive gene, which depends on the selection for breeding purposes of animals free of the gene. In scrapie there is no laboratory or other test known which will allow one to distinguish between the 3 genotypes. One is forced, therefore, to use the classical method of test-mating with other animals of known genotype, either grey or black, and keep enough progeny under observation for long enough. This is a cumbersome procedure with scrapie, as the progeny have to be kept for some 4 - 5 years, depending on the numbers and genotype of the dam. In practice it is only possible to test rams and these require to be mated with some 30 grey ewes to ensure about 20 female progeny being available at 9 months old for observation until they have had a sufficient chance to manifest the disease.

Of the rams tested so far, only 1, the ram SS of paper 15, table 2 has proved clear to a tolerance of at least 1 in 100. Many of these rams were not selected as being likely to be clear of the gene, and it is hoped that the current programme, in which greater care is being used in selecting the rams to undergo test-mating, may reveal more clear rams. In the meantime this clear ram is being used in an attempt to reduce the incidence of scrapie in sections of certain affected flocks.

The reaction of British flockmasters to the occurrence of scrapie in their flocks varies considerably, of course, but in the breeder groups with which we are working it is no longer considered to be an overwhelming calamity. In flocks with proper records in a closed breeder group, it has generally been possible to contain the disease to economically reasonable proportions, while instituting a long-term breeding policy control. Perhaps one of the major advantages of a joint attack on scrapie is that the owner and stockman no longer feel alone and isolated with a baffling and insoluble problem.

DISCUSSION

Dr. Mulhern:

Dr. Parry, how many cases did you say that you thought occurred annually in Britain?

Mr. Parry:

In my personal experience, I am seeing about a hundred cases, but I am dealing with particularly one population in which we know the disease occurs badly. The total number of Suffolk sheep is, I think, something like 30,000. Mr. Steele will speak to this. The disease is concentrated in our experience mainly in the best flocks. As I say, there may be 10,000, maybe 20,000 cases a year. I wouldn't know, but I would hazard it is something of that order. All I can say is my own home county of Wiltshire, which was ravished by the disease in 1820--if you talk to flockmasters and farmers whom I know personally from family connections, they do not know what I am talking about. They have never seen it. It has never been discussed within their memories.

This is very unreliable, unsatisfactory evidence, gentlemen. It's all that I can offer you. Perhaps others can offer more.

Dr. Carter:

In the sheep population in Wiltshire, are they largely Hampshires?

Mr. Parry:

They used to be, Dr. Carter. I regret to say they are declining, and they would represent I suppose 5 per cent now. We have mostly got Scottish sheep, Border Leicester x Cheviots.

Dr. Dickinson:

Just briefly with reference to the last question, I wonder if Mr. Parry would like to comment. We had a crude estimate of perhaps 400 cases per annum in the Suffolk breed in Britain. Does that seem a reasonable number in your experience?

Mr. Parry:

I would think this is perfectly fair, yes.

Paper No. 42

THE CONTROL AND POSSIBLE ERADICATION OF NATURAL SCRAPIE IN SHEEP. III. THE RELATIONSHIP OF SCRAPIE TO THE LIVESTOCK INDUSTRY.

H. B. Parry[1]

There are two principal situations in which a country's livestock industry may find itself; (1) The area is thought to be free, but scrapie has been recognized for the first time recently and (2) scrapie is an old established disease endemic for many years in the region.

The Newly Recognised Occurrence in an Area Thought to be Free.--This situation has occurred in the past 10 years in Australia and North America. At the material time little precise information about the dissemination of natural scrapie in the field was available. The laboratory evidence had demonstrated the presence of a transmissible agent in affected animals, which led to the inference that scrapie was due to a neurogenic virus with an unusually long incubation period. Prudence demanded that the disease be treated as an infectious one and that the veterinary control policies normally applied to exotic infectious diseases should be enforced; namely, the slaughter of affected animals and their

[1]Wuffield Institute for Medical Research, University of Oxford, Oxford, England.

immediate contacts, with the quarantining of live sheep moved from the affected premises and their use for immediate slaughter only.

Such a policy in the case of a chronic disease appearing in middle age can lead to a very considerable disruption of old established marketing practices and much hardship to certain sectors of the industry; it is also expensive to enforce in terms of manpower as well as money. It is only being applied in some 5 or 6 countries of the world at the present time; its effectiveness is difficult to assess, as figures for the incidence of the disease in the absence of control measures are difficult to arrive at except where the disease has occurred as an isolated focus of imported sheep, whose slaughter has removed the whole focus.

The present validity of such policies depends on the supposition that the transmissible scrapie agent is sufficiently infectious to ensure that a significant proportion of all natural cases of scrapie are due to dissemination of the TSA by infection rather than through the genic apparatus, and that the role of a recessive gene is of minor importance. The only available body of acceptable data relevant to the natural disease from closed breeder groups has been presented in papers 12, 13, 14 and 17, and this evidence is entirely against this supposition; on the contrary, it is difficult to evade the conclusion that, in those breeds and breeder groups for which adequate data are available, the occurrence of natural scrapie in the field is determined almost entirely by the genotype of the animal.

In countries where the disease is endemic or has occurred sporadically for many years.-- In Britain, where there has been no governmental intervention, the disease has been known so long, that the industry has learned to live with it. In such a situation any measures for alleviating losses from the disease have to be less radical and must meet certain practical requirements.

(1) They should not be more burdensome than the disease itself.
(2) They should disturb the general organisation of the industry as little as possible.
(3) They must command the general support of flockmasters and shepherds, for without their whole-hearted support any system of control is doomed to failure.
(4) They must provide sheep at least as good in production qualities and in flockmasters' esteem as those they remove as prone to the disease.
(5) They must involve the leading flocks, the principal suppliers of breeding animals, in a cooperative breeding venture which is best linked with the general improvement of the productive capacities of these flocks.

Bearing these requirements in mind, in Britain the best pedigree flocks have been enlisted in a voluntary scheme which would allow the co-ordination of efforts to control the disease on the existing policy of eliminating scrapie strains, but on a breed instead of an individual flock basis, while reducing other causes of loss of profitability. At the same time the scheme should provide as much epidemiological and scientific data as possible, and yet be in large measure financially self-supporting and independent, which would allow ready modifications in the light of experience. The Oxford Sheep Health Scheme was designed to meet these needs. The crux of the Scheme is the mutual confidence between flockmaster and shepherd on the one hand and the scientific advisers on the other, so that each party feels personally responsible to the other for the success of the individual flock and the Scheme as a whole. To obtain such a basis a scientific adviser must be in a position to give advice which is sound economically and practicable agriculturally, and to guarantee that the detailed information given him will not be disclosed to any other individual or organisation without the consent of the giver.

The organisation of small groups of flocks into collaborating breeder groups has made it possible to initiate an experimental test-mating programme for the presence of the s gene in a selected group of young rams, which are potential senior stud sires, while at the same time collecting data on other performance characteristics of their progeny which will permit a very much more detailed appraisal of the breeding abilities of such rams than is commonly practised at present.

The attitudes of British flockmasters with pure-bred sheep to the occurrence of scrapie in their flocks are various but may be summed

up thus. In breeds or pedigree flocks unaccustomed to the disease, there is initial alarm. Amongst those with experience the attitude is one of less concern, balancing the economic advantages (animals carrying the scrapie gene are often superior in many breed points and command a higher market price--see Paper 14) against the economic losses (from a higher ewe wastage rate); much can be done to contain such losses by good shepherding and flock management, so that competently managed flocks are not unduly concerned, although mediocre ones may be.

Those breeding commercial (grade) females for their own use or for sale, are concerned lest their sheep may get a bad name on the market, which is only likely to occur if the gene frequency in their flocks is high and they sell directly to other sheepkeepers.

Commerical flockmasters producing fat and feeder lambs for sale under one year old are usually quite unconcerned about the development of scrapie among their rams, or even their ewes if these are bought in, for in practice they do not consider contagion to be a real risk. They are perfectly prepared to purchase rams from scrapie affected flocks and their only complaint will be if a ram succumbs at too early an age. Indeed as there is evidence (see Papers 14 and 43) that the progeny of rams carrying the scrapie gene make better live weight gains than those of other rams of similar breeding there is a real tendency to prefer such rams as fat lamb sires.

REFERENCES

Parry, H. B. 1957. Scrapie and related myopathies in sheep: Preliminary observations on their investigation and attempted control by a voluntary Health Scheme. Vet. Rec., 69, 43-58.

Paper No. 43

THE CONTROL AND POSSIBLE ERADICATION OF NATURAL SCRAPIE IN SHEEP. IV. THE PRACTICAL PROBLEMS OF FLOCKMASTERS

T. W. Steele[1]

Firstly, may I take this opportunity of saying how appreciative I am to be given time at this meeting to be heard. When arrangements and details of this Seminar were made known at home, The British Sheep Society, of which I am a representative, decided my presence would be of value to present our policy and action in the work of collecting reliable field data and recording pedigrees and progeny information. Hence my appearance here this week.

It has been made possible especially by the Suffolk, Dorset Horn and Romney Flock Book Societies of England.

I would basically like to elaborate on the British Sheep Society; and the most relevant rule that we have is that the Society is formed for improving the breeding of sheep and for making the best use of records being kept by members relating to their sheep.

This Society, which was launched in July 1963 has taken in the original members of Dr. Parry's scheme which has expanded and improved through the last 12 years and it is now being made available to any flock in the British Isles to participate, providing they fulfill all the regulations which are laid down in the Rules.

[1] Flockmaster, Elmley Castle, Netherton, Worcestershire, England.

The charge to members will be graded according to the class of membership and the services received, and will be assessed annually on the basis of (1) a subscription, plus (2) a fixed charge for the first 50 breeding females and thereafter a per capita charge declining with the number of breeding ewes and depending on the complexity of the services provided and plus (3) a small fee for visits depending on the accessibility of the farm.

I am personally concerned with Suffolks and Dorset Horns, and so I have knowledge of immediate importance concerning Suffolk Sheep. I would like to put forward the economic factors relating to Suffolk Sheep with regard to scrapie.

First of all, I will give a rough outline of the development of the Society at home. We have heard during the week that Suffolk Sheep are particularly prone to scrapie, and during the last decade regardless of adverse publicity about this scourge we have had a phenomenal increase in the popularity of the breed.

In 1953, 21,500 ewes were registered in the flock book, and in 1961, 37,750 were recorded. I know for a fact that during the last two years we have received new members in the region of 150 annually, so I am sure when figures are published we can very safely assume that we have doubled the numerical strength of our Society.

The general procedure amongst flockmasters varies according to the general condition of their sheep relative to scrapie.

Flocks Where Scrapie is Present

The basic financial income of all flocks is realized from the sale of rams for pure and crossbreeding. Therefore this group of flocks concentrate on providing this type of sheep which can be done most successfully.

I have evidence from our own flock records that when rams from this category are compared with "cleaner" rams they show up most favourably in increased live weight gain of their progeny.

We bought two sons of a particularly successful sire who had attained a respectable age and we felt confident of his freedom from scrapie. These newcomers were mated along with two older and more reliably 'clean' rams to comparably aged ewes divided into groups giving preference to the two older rams for producing female replacements.

The resulting male progeny were taken after weaning at approximately 120 days and maintained under the same field conditions for about 6 weeks. The following weight gains for each progeny group were obtained:

		No. of Sons	Including No. of singles	Daily Gain
By one Sire	Ram A	21	2	.83 lbs/day
	Ram B	15	1	.73 lbs/day
	Ram C	15	3	.69 lbs/day
	Ram D	12	0	.66 lbs/day

Facts then came to light that the sire of Rams A and B was an affected ram, dying of scrapie at a ripe old age, whereas the reliability in regard to scrapie of rams C and D has improved to this day.

This must demonstrate the effect of this class of sheep, when used in commercial application to fat lamb production. Furthermore, the decreased life expectation is not of really significant importance as the farmer has acquired a highly predominant, fertile ram with a life of up to 5 years which to all practical purposes will fulfill his requirements and give him a good growthy bunch of lambs.

Ram B eventually manifested the disease and Ram A has not been used further in our flock, but has been loaned to Experimental farms for use in comparisons with other breeds and crosses. I might add that on the first year's results he came out top. He has also produced pure bred progeny from one ewe which were reared along with every other representative of our native breeds and they have proved the best in live weight gain and carcass appraisal winning 1st Prize.

The breeders in this group tend to slaughter their ewe lambs at the appropriate age as they do not wish to jeopardise the sale of their rams by bringing their name as a breeder into disrepute.

Flocks Where the Scrapie is Not Known

These breeders have a freer hand and tend to supply the majority of the females for flock replacements and new breeders.

Their rams tend not to be always of the same high standard as those of group A, but the females are much sought after. They have a greater number of females for sale as their annual wastage of ewes is not so high as in an affected flock.

The final point I would like to discuss, sir, is the steps for the control of the disease as practised by the flockmasters within our scheme.

A very great deal of confidence and respect has been built up in the organisation during the last 10 years and detailed pedigrees and full history of all sheep are being made available to other members on request.

The most progressive members have established groups of three or four breeders in their own district and we have four or five such groups in each corner of England. Each group and member has access to our "Chinese" white ram whom we look upon as the corner stone to the eradication of scrapie from our flocks. Ewes are dispatched to him for service at fees of about 140 dollars and previous offspring of his have been shared by groups. We have just had the second crop of these matings which have been most encouraging.

The first crop produced several very good rams, one of which was most successful in the awards at the Royal Agricultural Show of 1963 and considered by many breeders to be one of the best rams on show.

A good fall has resulted in the second crop showing the virile, active condition of this ram who is now 7 years old. This is a point of importance where breeding stock is concerned.

The resultant progeny are then taken and the best ram lambs have been mated with matron ewes over 5-1/2 years old to produce female replacements and potential flock sires. This class of old ewe are very prolific and produce good falls of lambs.

One of the main aims of the British Sheep Society is to establish a definition of standards of Stud Stock for home sales and for export which should assist and give confidence to purchasers seeking superior stock backed by closely supervised health and performance records.

This white ram has established his position in our breed and is the most sought after ram from his records and performance. He has proved most effective and left us with a surplus of progeny which will have considerable influence within the breed. These sheep will be made available to persons provided they are prepared to make them available to our scientific staff if required at home or abroad.

Crossbred ewes, sired by our "black" rams are being gathered together, along with similar genetical ewes from our pedigree flock, into a secondary testing flock. This flock of ewes will then be used to test mate our young sons of the white ram in order to establish their own genetical make up regarding this scrapie gene.

As you can appreciate, this involves considerable numbers of sheep and resultant progeny which have to be kept on until they attain old age. This is, however, of vital importance to our scheme and must be pursued very energetically to establish the next line of "white" breeding.

DISCUSSION

Papers 42 & 43

Dr. Gordon:

I may not be completely informed about this, and so perhaps you could give me the answer. In Britain the National Sheep Breeders Association used to look after the interests of sheep breeders--does this organization still exist?

Mr. Steele:

Yes, this organization still exists and in fact it is sponsoring the trials I referred to in one instance for assessing breed potential experiments of that nature. We do feel it has taken rather a backward view in this recording sphere within our group, and we feel we want to get on faster. No headway is really being made through the National Sheep Breeders Association.

Dr. Gordon:

The National Association is representative of many different registered breeds in Britain. What is the relationship of the British Sheep Society to the National Sheep Breeders Association?

Mr. Steele:

The British Sheep Society that we have formed?

Dr. Gordon:

Yes, the British Sheep Society. What is its relationship to the National Sheep Breeders Association? Is it affiliated?

Mr. Parry:

May I speak to that. This is not confirmed, but negotiations between our chairman and the president of the National Sheep Breeders Association and their council are, that they are going to nominate a member on our court and we are going to have a member on their council. So I hope that our relationship will be very close, Dr. Gordon.

Dr. Gordon:

I was wondering if the Suffolk Society are still members of the National Sheep Breeders Association.

Mr. Steele:

Yes, certainly. We haven't fallen out in any way, and we just want to sort of get moving a little quicker.

Dr. Gordon:

I was just thinking that as far as this audience, containing as it does a number of sheep farmers, is concerned, they may like to know that there is a National Sheep Breeders Association in Britain which represents many breeds.

Mr. Steele:

Some 80 members, I think, Dr. Gordon, at the moment, in 12 breeds.

Mr. Parry:

And we have at least two breed societies who are members and five others who have asked to become members and are awaiting ratification.

Dr. Gordon:

Could you give me the distribution of the breeds within the Society? Are the Suffolk the important ones?

Mr. Parry:

Yes, they are the majority.

Dr. Jensen:

Mr. Steele, I understood you to say that the progeny of the affected flocks show superior weight gains. Was this determined by formal feeding experiments?

Mr. Steele:

No. We are rather pioneers, in my opinion, in that I can't find out any information about weight recording other than what I personally do on my own farm. The point was that we require fresh blood in our flock. I saw progeny of an old ram which was particularly outstanding. I acquired two sons of this breeding, introduced them into my flock, mated them along with two other rams, making a total of four, with the older members of my flock, but they were actually put with inferior ewes because I wasn't requiring female progeny from these brought-in rams. But when we worked out our figures they had in fact given superior weight gains to the two older original rams.

Now, of course, we din't know that year the full implication of this. It is only when we look back and when we realized the position that these things come to light.

Mr. Parry:

Dr. Jensen, we have no ram testing stations or live weight gain testing stations in Britain. All we can do is contemporary within-flock within-year comparison under a roughly standard system of management. All the progeny are treated in the same way under farm conditions.

Dr. Hourrigan:

We appreciate, Mr. Steele, that when you have scrapie among these flocks a certain culling process takes place in an attempt to eliminate scrapie. It isn't clear to me how you make your decision to eliminate this animal or that animal when you are in that process of culling.

Mr. Steele:

It is fairly straightforward in our case, in that we buy rams when they are lambs. We probably use four or five in our particular flock. They are used, the progeny is assessed on its performance. They are then put into temporary cold storage, and when the ram reaches four or five years, he either dies or we find out from the flock of origin to give us a fuller picture, then we can take action

either to use the ram again or remove the original progeny from our flock. Does that answer it?

Dr. Hourrigan:

Let's say in a typical year you discover that several of his progeny have in fact scrapie. What do you do then?

Mr. Steele:

This ram would then be removed immediately from our flock, and in our particular case these ewes would be transferred out of the pedigreed flock and used for test mating, rising generations. But we actually haven't had cases of females going down with scrapie in our flock. It is always the rams we have bought, either through being affected or becoming known greys due to the parents, showing signs, and consequently we have removed their ewe progeny out of our main flock.

Dr. Hourrigan:

And then you sell your rams into the commercial flocks, his ram progeny?

Mr. Steele:

They would have been sold when he was used the first year, but the other point is that we have got a considerable trade with rams, and I think it is sort of general knowledge that they are one of the more difficult groups of livestock to maintain on the farm. We sell them as lambs, and they are effective when they are nine months of age, so that even if these particular rams were going to die of the disease they would still presumably have a fair chance of being used for three seasons, which I would suggest is a very average age in normal farms on the crossing basis to expect the rams to last. Being young rams, they are more active than when they get older, and general policy is to sell these old rams when they reach that age, whether they die of scrapie or not. So that it is not harmful to a man producing fat lambs if in fact this ram is going to die, because he has had his money's worth in most cases.

Dr. Dickinson:

I would like to ask you one or two questions relating to this suggestion you made about the growth of the animals. I was very critical, as most of you know, the other morning of the information which Mr. Parry presented on this particular point. I said it wasn't even evidence. What Mr. Steele has said now is coming much more within the realm of what one must treat as evidence, and therefore it deserves a more careful consideration.

I would like to make one or two simple points here. The first one just to get my own mind clear, you said you have been in fact collaborating in terms of some of the rams supplied, that you were using some other organization. They have been carrying out some lamb growth trials which involve Suffolk sheep.

Mr. Steele:

I have no knowledge on that point.

Dr. Dickinson:

Therefore, the question comes round to a statement about what constitutes evidence or how strong has evidence to be--and I am assuming you are talking here about early growth differences in the progeny of these animals, and how strong has evidence to be for it to count as genetic evidence in this particular respect? I happen to be a member of an organization which probably has done more on this particular issue in terms of more variety of work than most other places throughout the world, and our results concur in general with findings elsewhere that this early growth is subject to enormous maternal bias because of lactation variations, and partly because of this the genetic control is present only to a relatively low degree. Therefore, it becomes that much harder to establish that differences which you seem to observe are in fact really genetic. Now, this is a commentary on the question. Therefore, my question is this. I asked if you were associated with Thornber's work, because they have attempted controlled trials involving Suffolks, and two other breeds which I have forgotten now, and I have seen the manuscript accepted by the British journal, Animal Production--it is in press at the moment--and the conclusions from this reasonably carefully designed work in which I think they used six rams of each of these three breeds, was that at the end of the day they could conclusively show nothing with respect to early growth of the progeny and its genetic control.

So all I am saying here is that one has to be very wary on this particular kind of characteristic in drawing any conclusions hastily, but the kind of evidence which you are submitting this morning is much more within the scope of our consideration than anything which I have previously heard.

Mr. Yohe:

Mr. Steele, you mentioned your Chinese white ram. I take it that these are very rare in Britain, or is there just one of them? Are breeders willing to pay a premium for rams which they feel are either white or hope to be white, and if so how much in pounds or dollars and cents is this worth to breeders?

Mr. Steele:

The normal figure, the high figure, is just under $3,000, which has been established within the breed of recent years, and the particular ram that I referred to that was exhibited at the Royal Show, I would have been very happy myself to have given twice that much money for this particular ram. But we are breeding them within our own flocks, so that that's the sort of value that I place upon them, and I am sure a lot of other people do. At the moment a lot of people don't even have a chance to have them. We at the moment keep it to ourselves.

Dr. Carter:

I would like to pursue this matter of your testing for growth weight a bit further. You test your ram lambs in your test flock, and other Suffolk ewes that are suspect or for some reason put in there. Is that growth rate that you are testing from birth to weaning or to 120 days, or is it post weaning?

Mr. Steele:

The procedure of our weight recording is that the first weight we attach importance to is the birth weight. Then we weigh all lambs between 3 to 4 weeks to establish the possible milking ability of the ewe. Then weaning weight is taken to get a complete picture, which is about 120 days, in May, and these particular figures that I shall work out are related from weaning, for 6 weeks, when they are completely away from their ewes, all in one fold--that is, all these rams lambs--before we start preparing them for sales.

Dr. Carter:

That is post weaning?

Mr. Steele:

Yes.

Dr. Carter:

I have had similar experience with our own sheep, primarily Hampshires.

THE CONTROL AND POSSIBLE ERADICATION OF NATURAL SCRAPIE IN SHEEP. V. A REALISTIC PROGRAMME FOR THE CONTAINMENT OF SCRAPIE

H. B. Parry [1]

INTRODUCTION

Whatever the precise mechanism of the pathogenesis of scrapie may prove to be, there is a strong genetic component determining the clinical manifestation of natural scrapie in sheep. The data given in Paper 15 show beyond any reasonable doubt that in an environment entirely congenial to the development of the disease, the progeny of one ram will not manifest scrapie even when their dams are clinically affected during their gestation and suckling.

Thus in the breed there is a non-scrapie prone family within an affected flock, where the disease is prevalent to varying degrees in other families, which may be partially or completely scrapie-prone. The pattern of this proneness to scrapie is that expected of an autosomal recessive gene.

It is thus feasible to attempt the selection of animals for their non-proneness to scrapie and to build up within an affected breed families and strains not liable to manifest scrapie.

Under current British conditions, any programme must be self-supporting financially and rely on commanding the respect of flock-owners to an extent that they consider the programme sufficiently sound for them to underwrite it financially.

Such a voluntary self-financed programme has been operating in the United Kingdom for more than 10 years. It has developed in 3 phases. Phase 1 was the development of a recording system which provided accurate Vital Statistics to ascertain the position within any breeding population and to determine the pattern of occurrence of the disease therein. Phase 2 is one of restriction of the dissemination of scrapie-proneness by not retaining for pedigree breeding any animals with parents or close relatives affected. This may be expected to limit the dissemination of a recessive gene, but will not eliminate the heterozygous animals with no known affected relative. Phase 3 augments phase 2 by the use of test-mating procedures to determine whether stud rams are free of the gene.

BREED ORGANISATION

In any breed-registered flocks fall into 3 categories, (1) the elite stud or foundation flocks producing almost all the most desired breeding sheep and doing constructive breeding, but representing only some 2% of all flocks of a breed (2) Ordinary multiplying flocks producing some good rams, but mainly rams for use with commercial (grade) ewes (3) The least good flocks producing a few rams for commercial purposes.

The programme is aimed at class (1) flocks only, with an occasional class (2), for several good reasons. Class (1) have a higher standard of management and hence can sustain a more sophisticated programme, they influence profoundly the whole breed by the breeding stock they sell, and if the disease is brought under control in these flocks, it should be simple to control it in the other two classes. The impact of the programme on Class (2) is indirect through the example of Class (1) flocks and through the sheep bought from them. Class (3) and commercial flocks are neglected entirely.

[1]Nuffield Institute for Medical Research, University of Oxford, Oxford, England.

ORGANISATION OF ELITE STUD FLOCKS

The elite stud flocks of a breed are organised in breeder groups forming breeding populations of, if possible, 2000-3000 breeding ewes. Each breeder group is made a closed breeding population not going outside the group for breeding animals, except in exceptional circumstances or in a special portion of the flocks, as soon as possible; for practical economic considerations in the early years such groups can only be semi-closed for rams, although completely closed for females.

The records kept in these flocks (see Paper 10) allow after 5 years or so an assessment of the scrapie incidence within a breeder group.

THE GENERAL ASSESSMENT OF THE SCRAPIE STATUS OF A BREED AND BREEDER GROUP

Table 1 gives details on how this assessment is arrived at initially and kept under continuous review.

PROGRAMME FOR FLOCKS OF BREEDS IN CLASSES SC1 AND SC2

As scrapie is unknown for practical purposes in these breeds they will have a very low presumptive frequency of the scrapie gene with certain breeder groups probably entirely free. Thus the immediate aim is to keep these breeder groups as closed breeding populations under close supervision so that should any case of neuromuscular illness of a scrapie-like kind occur, it will be immediately recognised. Later experimental test-matings of some rams will be made.

PROGRAMME FOR FLOCKS OF BREEDS IN CLASS SC3

In these breeds scrapie is endemic to a greater or less extent with some flocks affected and many not. In all flocks a phase 2 programme is used. Any affected animals are sent to the butcher as soon as is practicable, while their progeny and parents and other

Table 1.--General assessment of scrapie status of Breeds and Breeder Groups

Class	Occurrence 1952-64				Remoter memories	Assessment of scrapie status
	Supervised breeder groups in sheep			Other reports by reliable witnesses		
	Home	Bought	Sold			
SC 1	0	0	0	0	0	Breed completely clear with presumptive gene frequency zero.
SC 2	0	0	0	1-2	uncertain	Breed probably mostly clear with presumptive gene frequency in supervised flocks very low but gene present elsewhere.
SC 3	+		+	+	+	Breed affected with some flocks affected severely and many free. Presumptive gene frequency very variable, high in individual flocks.
A	+	+	+			
Individual flocks B	+	0	+			
C	0	+	0			
D	0	0	0			
Testmated E	0	0	0			Declining gene frequency becoming negligible in three generations.

close relatives are not used for pedigree breeding; they may be culled if this is economically justified, or they may be retained in a section of the flock not used for breeding flock replacements or in a subsidiary flock. By voluntary agreement these animals are not made available to other breeders, except that the rams are sold for crossing, i.e. commercial fat-lamb production.

As soon as it is possible to organise a closed breeder group with adequate recording and a good prospect of stability of the flocks' organisation for 10 years ahead, a programme of test-mating selected young rams for absence or presence of the gene is put in hand. In practice it is not feasible to test mate females, as it would require the progeny from more than half of her reproductive life to provide sufficient numbers of offspring. It is necessary therefore to work through the rams, which are, of course, genetically speaking by far the most important element in the flock.

When a ram has been proven clear to a tolerance of at least 1 in 100, he and his progeny are used to build up a low scrapie prone family or families within the breeder group by mating him with ewes thought to be probably white, i.e. over $4\frac{1}{2}$ years old and no close relatives affected. If proven or Chinese White rams can be used in such a family for 3 generations, the scrapie gene frequency should be reduced to such a low level that the disease will cease to be of any economic importance in the sheep stocks derived from matings of the F3 generation.

TEST-MATING PROCEDURE FOR THE SCRAPIE GENE

As there are no laboratory or other methods available by which it is possible to identify the 3 possible genotypes of a simple autosomal recessive gene (see Paper 14), the classical method of mating a male of unknown genotype with females known to carry the recessive gene, either the homozygous recessive or the heterozygote is used. In scrapie it is not practicable to assemble enough homozygous recessive (black) ewes, because their expectation of life is too short (1-3 gestations) and they are as yet too valuable for other research purposes. The heterozygote (grey) ewes have therefore to be used and this requires 30-35 ewes to be mated with each ram for a test, and the progeny kept for 3-5 years depending on the number available. The clear test will normally take 5 years, so that a 'Chinese White' ram will be $5\frac{1}{2}$ years old before he can be widely used, although he may have been used as a speculation in previous years.

The requirements of such a test-mating programme are briefly as follows:

(1) A special breeding stud of 'black' sheep to provide a supply of 'black' rams for the breeding of 'grey' ewes.

(2) Procurement of 'grey' ewes by mating 'black' rams with suitable ewes. In general the grey ewe will be a cross-bred with commercial qualities suitable to the region in which she will live, but some purebred ewes will also be available, the progeny of spontaneous cases.

Selection and mating of rams with 'grey' ewes.--The rams will be selected as outstandingly good specimens of their breed, bred from parents thought to be white. They will initially be mostly the sons of any Chinese White ram, but rams of other strains will be tested to reduce the risks of in-breeding. The rams may be selected when 9 months old, but preferably they will be 21 months when their first crop of lambs will be available for an initial assessment of their breeding capabilities.

The mating of these rams with grey ewes will be under strict supervision.

Progeny Observation Groups.--For each ram - 'grey' ewe group, the progeny will have to be kept under observation for $4\frac{1}{2}$ years on average unless scrapie develops in the group earlier. In practice it is more economic to keep the female progeny only and to sell the male progeny as lambs for slaughter. About 20 - 25 female progeny will be a desirable number and this will require on average about 35 ewes put to each ram.

These female progeny groups will be used as commercial ewes for fat-lamb production while they are under observation, which will greatly reduce the cost of the test-mating procedure.

The provision of such an integrated facility demands some considerable organisation. This has been built up for one breeder group in the United Kingdom during the past 2 years and is now under the direction of the Sheep

Development Association. It is being linked with selection for other economically important characteristics.

THE ROLE OF A SLAUGHTER-QUARANTINE POLICY

Under British conditions at the present time the general imposition of any such policy would be a disaster. Firstly it would destroy the epidemiological evidence being collected. The position of Britain as a collecting area for such data and for general Vital Sheep Statistics is unique, as the geographical location and density of the sheep population allows close contact and frequent visits by a small number of observers, while the organisation of the leading flocks under the auspices of the Sheep Development Association provides a stable base for the collection of standard records under independent supervision.

Secondly, the slaughter of all animals in a flock in which a case of scrapie occurs will inevitably include the slaughter of 'white' and 'grey' animals. If it is an isolated first case, the odds are that many more 'white' animals will be slaughtered than those carrying the gene, and it is these 'white' non-scrapie prone animals which should be retained for breeding.

EXPERIENCE FROM OTHER BREEDING POPULATIONS

The only substantial body of data regarding natural scrapie in sheep in the field has been collected in one breed population in Britain, although limited data are available for a second breed.

It seems most desirable that comprehensive data similar to that for the first breed population should be assembled for other breed populations in Britain, and also in other parts of the world.

The Sheep Development Association is anxious that breeder groups employing similar systems and standards of recording and supervision should be set up in various parts of the world, not only to provide comprehensive epidemiological data on scrapie but also to build up a reliable pool of genetic material from which animals of different family strains could be selected for use in our own improvement programme in Britain.

DISCUSSION

Dr. Dickinson:

First a correction, please.

Parry's opening remark was something to the effect that I had said that the scrapie agent must be ubiquitous. I never said any such thing. I think the transcript will show that I have used the word ubiquitous prior to this morning on only one occasion. I was reading from my script at that time, and I will reread it to you now.

"There seems little doubt that when two affected Suffolk sheep are mated, their progeny almost always developed scrapie within 4 years. The same applies where one or both parents manifest the disease a year or two after the progeny are born, but with a disease of prolonged incubation, like scrapie, there is hardly any point in distinguishing this class from the former until more is known of the presumed infectious period. If a disease of short incubation period shows this property of always appearing in progeny of affected x affected matings then either the pathogen must be highly infectious and ubiquitous or some mechanism for its nongenetic transmission from the parent to the progeny would have to be postulated."

I used the word "ubiquitous" in connection with another model entirely, so I cannot be going down in the record as having said what I think you suggested that I had said.

Secondly, it would seem to me that the evidence presented so far this week has made the most tenable hypothesis so far as natural scrapie is concerned, that in which contagion must be allowed for.

I have in many cases now been involved in experimental work which is identical in effect with test mating of rams. It wastes a lot of energy if you are in fact test mating for a situation which does not exist; and it would seem to me to be only relevant to test mate in this particular circumstance we are in of understanding scrapie at the moment if you test mate in a situation where you can be sure that there is exposure to the scrapie agents, plural, which you consider that in that context you are interested in because test mating prevents you from doing other things such as selecting for the things you are ultimately interested in as a commercial

breeder, fertility, growth, wool, meat, et cetera.

Dr. Stuart:

I would like to ask Mr. Parry what the purpose or the intent of the selective breeding program is that he is using. Is it the purpose to control scrapie or eradicate it, to work out a system of control or eradication?

Mr. Parry:

It is our hope to control it, Dr. Stuart, to reduce it to economically insignificant proportions. We have no illusions that we shall eradicate it because it is immensely difficult to eradicate a recessive gene from the population.

Dr. Stuart:

How long do you think it will probably take you to determine whether you will be able to control it by using your method? About how many years do you think it might be?

Mr. Parry:

I think in 5 or 10 year periods. I have told my flockmasters that they have to bear with me for 10 years.

Dr. Mulhern:

Mr. Parry, in a program in this country I think it is very beneficial for us to try to sort out hypotheses from evidence that proves the point, and in our 3 days discussion here there are hypotheses that have been expounded upon, and it is well for your sheep industry to recognize that these are more or less brought out to show a possibility that these conditions may exist and that they cannot be accepted until it is proven by evidence that they are acceptable. Otherwise we may bring confusion into the program that any approach based on the evidence that we have is the wrong approach because of these other hypotheses.

Now we come to the muscle question which I don't like to bring up again, but it needs to be evaluated relative to how often it does occur. If your point in mentioning it was purely scientific and you only occasionally find this, then, can you substantiate the number of animals examined and how often you find these lesions. This will give the group here a method of evaluation on the frequency of its occurrence and its significance from a diagnostic standpoint.

The second point is on the recessive gene. The other group has presented evidence for 3 days to challenge your hypothesis. My point here is that for our industry we must recognize that the recessive gene as a means of carrying on or perpetuating scrapie is not a proven fact, and you say that maybe in 5 or 10 years you will be able to show this, or at least you hope to--is that right? You seem to have some doubt about that point.

Mr. Parry:

I was asked about this particular test mating program for the control of scrapie, and I mentioned 10 years.

Dr. Mulhern:

I mean are you saying that you have proven that recessive gene is the mechanism by which scrapie is being perpetuated?

Mr. Parry:

You can never prove anything scientifically, Dr. Mulhern. All I am saying is that up to Monday morning, I wasn't fully aware of the evidence which has since been presented. I hope I made it clear that the program that I have just outlined is the one we are operating, having arrived at that decision prior to this meeting; and I hope I made it clear that this only applied as far as I was concerned to Britain and the breeder groups in which I personally am associated.

Dr. Mulhern:

Yes.

Mr. Parry:

Is that fair?

Dr. Mulhern:

Yes, and my only point here was to get an appreciation for the U.S. program, that must be more or less based on the evidence as exists today, and that evidence is the key to it, and that your point of the recessive gene is something that we should be looking at very aggressively for possibilities, but that the evidence as of today doesn't prove this point--is that true?

Mr. Parry:

No, neither proposal is proven.

Dr. Mulhern:

Which is the other proposal?

Mr. Parry:

There is maternal transmission. It is not proven fully in the scientific sense. We must go on and see. It may be proven in artificial environments, if I understand correctly what I heard yesterday. As you said yesterday afternoon, the crux is what is going to be the situation in the natural disease in the field. This is what I am hoping to unravel in my breeder group.

Dr. Mulhern:

This is another point that I wanted to bring up. There have been statements made that the artificial disease is different from the natural disease, and what are the facts to prove this point that they are different. One side says we reproduce it and it is identical in its reproduction, you generally accept that for proving this point; and yet what about the other point of proving that it is different.

We say well, you have no facts to show under natural conditions that the two diseases are different--but we have never before been willing to accept information that we have gathered under natural conditions as a basis for arriving at satisfactory evidence.

Now your scheme in England has given us a different approach to try to get more reliable information. But that information has to be weighed against the weaknesses, if they exist, in your scheme, based on research procedures that have been accepted for centuries.

Mr. Parry:

The question of whether the artificial disease is different from the natural disease must be a matter of opinion amongst yourselves and--all I would say is that I personally have a strong reservation in the back of my mind on two counts. One is that I have failed to reveal spread of the natural disease in my breeder groups. This may be due to some defect in my data.

We are continually looking for evidence of spread and we have been looking for this for the last 5 years, because after all, I was as interested in shooting down the lethal factor hypothesis as anybody else because I shall get it from my flockmasters if I don't come clean.

The second point is that in spite of the fact that all my field data as shown by Mr. Draper in that large table of sire progeny groups, in one group in which the big SS ram at the bottom was white, no cases of scrapie have occurred. This appears to be at variance with what is now beginning to show up in my experimental flock at home in which I did deliberately, I am afraid to say, introduce artificial cases with my natural genetic cases, and the answer is not the same.

It is much too early to say what is going to happen. You see, it is only in the last week or at least the last month that a case has been sufficiently clearcut clinically and these have not been examined pathologically; they may be different. I have just a doubt in the back of my mind, and I am wondering whether in 12 months time or 18 months time if I may not find myself very close to Dr. Dickinson on this.

Now I am faced with a proposition how can I reconcile the data I have at home with the data I am collecting in my natural flocks on farms. The first thing that comes to mind at the moment is that the artificial disease is introducing a slightly different effect. There is some evidence to support this in my results of whole body irradiation of new born lambs, which affects the development of the artificial disease, and I have merely one case of what looks like the natural disease which was irradiated, and it is coming up with what appears to be the natural disease.

This may be quite wrong, gentlemen. I may want to retract or alter this entirely. All I am saying is for me as a person and for my flockmasters we shall continue to have a reservation for the present.

And may I not fail to answer the muscle question, this is largely an academic question. From a practical point of view I would think that we might forget about it.

Dr. Mulhern:

Yes. That is what I concluded also. But, Mr. Parry, the only reason for my trying to clarify this point was to point out the importance of trying to separate what we have as evidence that we can accept and support

against what is opinion, and only time will tell us whether this is accurate or not.

Mr. Parry:

I am only telling you what we are doing.

Dr. Mulhern:

Yes.

Dr. Dickinson:

I thought this word "proof" would start getting bandied around. Let's have a standard of reference for this word "proof" for what it means in scientific terms. In terms of the criteria, and they are accurate, which Parry was adopting then, the theory of the evolution is not proved. That I would suggest to you for use as a standard of reference for the word "proof."

My second point, and this is a direct question--I will explain what the question means--what changes in advice to your flockmasters or what changes have you considered since March 1963 in terms of alteration of the advice you are giving?

I say this because--and here I have the script I used in my hand at Brighton March 1963 when I said identical things to those that I said yesterday to Mr. Parry and Mr. Draper in the audience except for one thing. I didn't put any of the contact information which I wrote on the board yesterday. That was not there. All the rest of the Suffolk breeding data, all the rest of our purchased sheep data were there. It was presented to him then. He must have understood it because Mr. Draper included as a footnote in a paper which he had--an inclusion which said something to the effect that further evidence has come to light, and this meant my Brighton talk. I don't know what "further" meant. I think he should say evidence came to light unless they knew of some before. Evidence has come to light recently.

Now Draper put that in because of my Brighton talk, and he obviously hasn't persuaded Parry of this, and Parry has perhaps not reconsidered the advice he is giving to flockmasters.

I ask a question, therefore, have you during this last almost a year started considering whether your advice to flockmasters ought to be changed or not?

Mr. Parry:

No, like Mr. Draper, I found myself confused; and as I wrote to you a little while ago, we have been waiting to see this paper in print, so I, who am an amateur in this, could look at it more closely.

One doesn't change a general pattern of advice to flockmasters quickly or without very careful consideration because this disturbs an established system. That is, I think, my side of the question.

Mr. Draper:

I just want to say one thing, that I am largely in agreement with what you say except that while what you said at the Brighton meeting was enough to stir up doubts, as it must have stirred up doubts in people's minds yesterday, that was the first time I had seen any of this.

If I remember rightly, you said very little about this in explanation. We were supposed to take it on authority. We have never seen any script of this at all. You went through it at a considerable pace, and it was enough to throw doubt, but I would have liked to have seen it more.

Dr. Dickinson:

The word "further" evidence was used in his footnote. So I ask what the other evidence apart from what I had presented was in Mr. Draper's mind on the issue of contagion. What did the word "further" mean in further evidence?

Mr. Draper:

I don't remember the word was "further." This was the sort of evidence we were discussing right at the beginning of this 3 day meeting, the evidence that is uncontrolled, the evidence of Greig which may or may not be reliable. This was the sort of thing I was thinking of.

Dr. Johnson:

Mr. Parry, I believe--I prefer to believe anyway--that you made a slip of the tongue. I believe that you said nothing is ever proved by science. Surely you didn't mean that.

Mr. Parry:

Yes, all you can do, Dr. Johnson, is to build up evidence towards one way which satisfies

reasonable men. But I have been taken to task by one professor at Oxford who maintains you can never prove anything, hence perhaps that is why I said it. But I am not a philosopher.

Dr. Gordon:

Mr. Chairman, we have been listening for 3 days to much evidence about possible ways in which scrapie spreads. On the one hand Mr. Parry has stated that scrapie is transmitted hereditarily and is never naturally infectious. On the other hand I have produced evidence to indicate that spread by contact at pasture cannot be excluded. Stamp and colleagues have also experienced spread by contact in sheep and goats. Nevertheless I think there is good field evidence to say that scrapie spreads in family lines (vertical transmission) in a high proportion of cases. A much smaller proportion in my view represent lateral spread at pasture from one affected family to susceptible animals in another. If I had to put percentages on this I would guess that over 90 percent are probably vertical transmission and under 10 percent lateral spread. Now Mr. Parry has provided much evidence that the disease never spreads by contact so if he is convinced about this I am going to propose to him a plan for eliminating susceptible sheep from a flock and if he is satisfied that the disease is not infectious there would be little risk in it. Mr. Steele has been building up a flock free of scrapie by the use of presumed homozygous scrapie-free rams. Such animals should be resistant to scrapie; but to ensure that no susceptible animals remain, inoculate the whole flock with scrapie infective material and remove any animal that becomes affected. In this way, a completely resistant flock would be built up and I would seriously suggest such a plan were it not for the fact that I would be afraid of creating carriers of infection that might be a risk to other flocks with susceptible animals.

If this policy were adopted it could teach us a lot about eradication of the disease because if, as is contended, it never spreads laterally, Mr. Parry need not be afraid of letting the agent loose and those with doubts about his hypothesis would obtain evidence that either confirmed or allayed their fears.

Mr. Parry:

Will you finance it, Dr. Gordon? I mean this is a real point.

Dr. Gordon:

Why?

Mr. Parry:

But who is going to do it? Who is going to stand the risk to Mr. Steele?

Dr. Gordon:

Your opinion of the situation is going to stand the risk.

Mr. Parry:

My opinion?

Dr. Gordon:

Yes. If he has no susceptible sheep then in your view there should be no risk.

Mr. Parry:

I made the point I think that there is serious doubt whether the post-inoculation disease is exactly similar to the natural disease. I wouldn't chance my neck on your proposition because I don't know whether your inoculation has any bearing on my genetic situation with whatever agent you like, say for argument sake, we will use the Suffolk agent in Suffolks. Is that fair enough?

Dr. Gordon:

You don't even need to do that. I can supply you with scrapie agent from 24 different breeds of sheep, including Suffolk, and you can mix the lot. If this is applied, nothing but sheep that are resistant to 24 different test products will remain, and if there is no risk of the infection spreading laterally then a very valuable flock would be established.

Mr. Parry:

Could I answer? We will take it, the agent will be supplied by mutual agreement. We know nothing about the susceptibility of blacks, grays or whites in the Suffolk breed to artificially induced scrapie. There is point one. I wish I did. I haven't had enough blacks to test.

All I have done--and I did show a slide--is that I have put it into three

other breeds, in which we don't think the disease occurs; and they would be, as far as one can tell, free of scrapie, but not test mated free, and these are susceptible to age 3 ½, and some do not go down. And in view of what Dr. Dickinson said about incubation period I don't know how long we should have to keep them under observation to establish a valid test.

I agree, Dr. Gordon, that this should be done on an experimental basis somewhere. I would be delighted to do it, and I am sure my flockmasters would allow me to assemble such sheep, but I could not recommend it to be done on any flockmaster's flock because he has got 25 years reputation behind him and how do you estimate the equity in this if I destroy the whole of his future prospects.

Mr. Yohe:

I asked the gentleman on the other side this question yesterday, and I would like to ask it of you today. Let's forget about proven facts because if we have to wait for proven facts we will not have any kind of program in the United States. This afternoon and tomorrow those of us on the advisory committee must make certain premises and proceed from there. So what I am asking is a premise, not a proven fact.

If you were in our position would you proceed on the premise that scrapie can be transferred by contact or would you proceed on the premise that it cannot be spread by contact, because each of us in our own minds must make this premise before we can make recommendations.

Mr. Parry:

Mr. Chairman, as I told you, I did not wish to give an opinion relative to the position in North America. I have been put this question. I am perfectly prepared to answer it if it is clearly understood that I am not wishing to offer any opinion whatever on what is appropriate in North America. Is that understood?

Dr. Anderson:

Yes.

Mr. Parry:

I will answer it in this way, Mr. Yohe, that based on my experience of scrapie in Britain in the breeding population with which I have been dealing we consider that in the field there is no risk whatever of contagion by contact, contaminated surroundings or residual contamination. We think on the existing evidence that susceptibility to clinical scrapie is entirely genetically determined, or so nearly entirely that for practical purposes other factors may be disregarded.

Dr. Gordon has suggested that ten percent might be an estimate of natural contact spread and 90 percent through genetic resistance or susceptibility, call it what you will.

On the data that I have handed to Mr. Draper, he tells me that the chances of other than the genetic method of spread are less than one percent on our data with the natural disease in the field.

We think, therefore, and I have so advised my flockmasters in a document in July 1962 that we think it is a reasonable practical risk to assume that scrapie is entirely genetically determined, and that we may disregard for practical purposes spread by contagion, not prejudging the issue whether or not contagion may exist and may in fact prove to be a significant part of the total method of spread. But at the moment we can see no evidence in natural scrapie that it will account for, putting it at its very worst, five percent of cases.

I would like to go on, if I may, and say--because it is only fair to reiterate for the benefit of the audience--that much of this depends on the examination of these nine sire-progeny groups in one flock with a high incidence and on this one proven white ram. It is the only white ram we have--this is a weakness, and I must stress it because it is only fair to add that this may prove to be an exception, and until we can find some more proven white rams this is a weakness in our case.

Mr. Yohe:

I want to follow this up. You mentioned this in Britain so we will take Britain. Let's postulate that scrapie is only in the Suffolk breed in Britain. Do you believe this strong enough that you would risk spreading it to the other breeds in Britain?

Mr. Parry:

By letting them come in contact, and so on?

Mr. Yohe:

Yes, by contact. Do you believe this strong enough--let's assume that no other breed in Britain had it. Do you believe this strong enough that you would take the risk that there is no possibility of transferring it over to the other breeds, that you would take this risk yourself, because that is virtually what we will have to do if we follow your line of thought.

Mr. Parry:

I would take this risk absolutely.

Mr. Yohe:

Thank you.

Mr. Parry:

We are taking that risk, Mr. Yohe. We have evidence that the risk as far as we can ascertain does not arise. It doesn't mean to say it may not arise with other breeds, but in--I must qualify that--in those breeds in those situations in which we have evidence we can find no sign of spread yet.

Dr. Stamp:

This is in context with your question. I think we must be very careful, Mr. Parry, here. In the first statement you made you said this applied only to your own environment. The second question that was asked was not concerned with your own environment. It was to the environment of every sheep breeder in Britain and on every farm in Britain. Do you appreciate the significance of that?

Mr. Parry:

No, no.

Dr. Stamp:

This is what you stated.

Mr. Yohe:

This is what I said.

Mr. Parry:

Oh, look here. No, I am sorry.

Dr. Stamp:

This is the inference I am sure that went over to the meeting.

Mr. Yohe:

I don't want to put you on the spot, but in essence this is the spot that each one of us finds ourself in, and the question I would like to ask of you is that if at present in Britain--you said you prefer not to deal with North America--if in Britain the disease were confined to the Suffolk breed do you believe strongly enough that it is not spread by contact that you would risk spreading it to all the other breeds of sheep in Britain by some type of program which would allow contact with infected sheep, because this is essentially what everybody here must decide for the United States. Now I won't ask you to decide this for the United States, but would you--

Mr. Parry:

May I slightly change it? You see, I have only certain limited experience. I do not know--

Mr. Yohe:

But you have had more experience than I have.

Mr. Parry:

Yes. All I can say is until I had assembled experience and data to the contrary with any breed with which I came in contact for which I have not yet adequate information I should assume that there was no risk.

Mr. Yohe:

And you would take this risk?

Mr. Parry:

I would take this risk if I were in practical farming, or if I get a newcomer coming into my scheme they will be told that there is in my opinion no practical risk at the present time.

I say this because my flockmasters and I are always prepared to review this situation if evidence comes to our notice--you understand.

Mr. Yohe:

I understand. Tomorrow you might think differently, but as of today you would be willing to take this responsibility.

Mr. Parry:

Yes.

Dr. Dickinson:

Might I comment on the exchange that was going on between Dr. Gordon and Mr. Parry a short while ago, that there was some present

practical issue in terms of control measures in Suffolk sheep using challenge.

Now we want to start on the basis of evidence. I would like to know anyone here--and I will amplify this when I have an answer to this one--I would like to know anyone here who knows of a case of induced scrapie in Suffolk sheep which appeared at under 2 years of age. That is that it could not have been confused with natural scrapie. Does anybody know, except in Dr. Gordon's group where he challenged them with the Cheviot agent, but the Suffolk Sheep Society in Britain would not acknowledge the only animals that went down in that group as being purebred Suffolks. Does anybody know of a single example, and I am going to quote one to you because nobody knows of this except Dr. Stamp and myself. Does anybody know a single example?

Nobody?

So is it sensible to try to transfer information which is being got out of the Cheviot breed with Cheviot agents to Suffolk sheep and Suffolk agent, whatever these might mean at this time? There is no evidence to start here, and this is the point we keep making.

There are agents. There are scrapies. Let's talk so far as practical control measures are concerned in terms of at least one factor known as a starting point, and we have not any in this case.

I will put my one case now. It is not pathologically confirmed. We ourselves have been trying to make Suffolk sheep go down with Cheviot agent. We have failed until 3 weeks ago. It is not confirmed pathologically. But in my own mind, I think in Dr. Stamp's mind, on looking at this one animal it has got scrapie of a very classical type, and it is at the present I think 8 months old.

This animal was challenged I think 6½ months ago subcutaneously with the SSPB/1/24 agent, the Cheviot agent. This animal has a control full sib unchallenged on which we have no comments to offer. It is unaffected. But this pair of animals happens to have both parents affected with natural Suffolk scrapie.

So here at long last to our minds we have got a break; we can potentiate this agent which is producing natural scrapie in Suffolks. We have it in evidence in one animal. We have only got two of this kind inoculated so I want these remarks to be taken in this light. But it looks as though the agent which is there already--the Suffolk agent which is going to produce scrapie we know for certain in these sheep (we have a sib to say it did it at a later date.)--We have evidence that the Suffolk agent naturally present in that sheep can be potentiated by some other agent, and this is the only example. There has been complete silence here when I asked for another example of induced scrapie of any type whatsoever in a Suffolk purebred sheep at under 2 years old.

I would like to work on the basis of my evidence or other people's evidence, but I don't like hearing people work in the face of no evidence.

Dr. Stamp:

Just one comment I would like to make on something that Dr. Gordon said previously, and this was about the frequency of maternal transmission and contact transmission. Let's be fair about this because this is going to be important in your discussions. There is no evidence of any kind to show what these ratios are. I think it is quite wrong to suggest that we know these facts.

Dr. Gordon:

I was making a tentative estimate.

Dr. Stamp:

Yes.

Dr. Gordon:

You can make this percentage suit your own estimate.

Dr. Stamp:

All right, I have made my point.

The second point is that I would suggest that the degree of contact transmission might vary from farm to farm and from area to area depending upon the weight of infection. It might also--and I would stress this, since it might be concerned with the question that was asked a moment ago by Mr. Yohe--lateral transmission may in fact, if the Icelandic evidence is accepted, be determined by whether there is a biological transmitter or not.

Mr. Yohe:

I would like to ask Dr. Gordon when we are talking about contact need we differentiate between contact with sheep showing clinical

symptoms of scrapie or contact with sheep which may eventually show clinical symptoms. That is, does the stage of contact of the animal need to be considered or are you willing to accept any contact?

Dr. Gordon:

There is no exact evidence at present as to how the agent passes from one sheep to another. On the evidence that has been produced the majority of cases appears to follow family lines. A small minority may occur by contact. How this happens we just don't know. When it can happen we just don't know because we have never found the agent in secretions or excretions of the sheep.

In reference to the pressure that you are placing on Mr. Parry to answer if scrapie is occurring in one breed can it spread to other breeds, and if it does how does it cross the gap between one purebred and another if it is only transmitted hereditarily. From British experience, and Dr. Stamp outlined this yesterday, the answer is, yes, it can cross the gap but, in America, the eradication programme appears to be preventing this. In Britain the disease has waxed and waned in different breeds over long periods of years and it must cross the gap between breeds either by interbreeding breeds or by contagion. I have had discussions on this question on many occasions and a typical example was one with a past president of the Suffolk Society which went something like this question. How do you think scrapie was introduced to the Suffolk breed? Answer: from half-bred sheep brought from Scotland where the disease was prevalent to the eastern countries of England. Question: Did the half-bred infect the Suffolk by interbreeding? Answer: No, the Suffolks were registered sheep and could not legally be interbred with the half-bred. Question: If it wasn't by interbreeding how do you think it crossed the gap? Answer: It must have been by contact. This of course is opinion; it cannot be accepted as evidence.

Mr. Yohe:

Wouldn't this suggest, however, as I have gotten the discussion here, that your Cheviot type scrapie is one thing, the Suffolk type is another, perhaps the Swaledale is a third type. Wouldn't this suggest that actually if this went from one breed to the other through contact that this must have occurred a considerable time ago, at least long enough that you have gotten modifications of these strains in the sheep?

Dr. Dickinson:

The evidence we have on this point is that transfer occurs--it is hard to transfer the first time, but once the first transfer is achieved the subsequent ones are easier.

Mr. Yohe:

You mean all the changes occur in the first generation?

Dr. Dickinson:

Virtually all the change happens the first time. This wasn't quite so when it was transferred from Cheviots to Welsh sheep to goats. But that wasn't very well controlled, that information, so it might take as long as three passage generations. But I don't think we have any evidence to suggest it takes longer than three passage generations.

Mr. Yohe:

When you run this scrapie now, this inoculation--and I don't want to get into the difference between natural and the inoculation--in the inoculation cases where you took the Suffolk agent over to Cheviots, and they displayed Suffolk symptoms and this was the first passage, you haven't carried this on to know whether or not the second or third passage will show Cheviot?

Dr. Stamp:

Not in the sheep.

Mr. Yohe:

Just one more question. When the goats came down due to contact, or presumably due to contact, had they contacted sheep that showed clinical symptoms of scrapie or were they merely with susceptible sheep that showed clinical symptoms later?

Dr. Stamp:

Both types of sheep. We actually tried to put as much infection into the pen as we possibly could, and all stages of the disease were used. Some sheep we put in we were

pretty sure were going to go down on family grounds because their mothers and fathers had the disease. Some were early clinical cases and some were late clinical cases.

Now I think I would agree with Dr. Gordon, I don't think there is any evidence whatsoever to answer your first question. All one can say is that if one accepts scrapie as a virus infection then presumably there is danger other than in the clinical case. This is not factual for scrapie, it is merely deduction from the evidence of other virus infections.

Mr. Yohe:

In the United States we have had outbreaks in both Suffolks and Cheviots. Does anyone know whether or not the clinical and necropsy evidence were different in these two breeds? That is did we seem to get two different strains here?

Dr. Stamp:

I could perhaps answer this question and say it could not possibly be so because the work I was reporting yesterday is quite recent work. I don't see how you could possibly have done this. I mean only we could have done it.

Mr. Yohe:

In other words, you don't know whether the Cheviots have a Cheviot strain corresponding to what we have heard in Britain or whether they had a Suffolk type?

Dr. Hourrigan:

Well, as a point in fact, as you will see in a slide I will show when I speak later, the Cheviot flock which was the source of the last three Cheviot outbreaks had in fact previously had a large number of Suffolk sheep moved through it from an Indiana flock found to be infected. Two of the Suffolk sheep--both rams--which moved from the infected Suffolk flock into and through the Cheviot flock in Illinois were showing signs of scrapie only 4 months after moving on into a Missouri flock.

In the combination Cheviot-Suffolk flock in Illinois only the Suffolks were slaughtered, not the Cheviots. Later on, about 4 years later as a matter of fact, Cheviot sheep moved out of that flock were found to have scrapie in three different flocks, one in the State of Oregon and two in Illinois.

Dr. Gordon:

Mr. Chairman, the question might be answered if the brain material from the Suffolks and the brain material from the Cheviots had been put away in formalin by someone in some laboratory, and if so this material could be sent to Dr. Stamp and Dr. Zlotnik might undertake a diagnosis as to whether it was Suffolk scrapie or Cheviot scrapie. Would that be possible?

Dr. Stamp:

Yes. We could always put it into mice as well. I think scrapie would come up even though it had been in formalin.

Dr. Anderson:

Dr. Hourrigan, do you know whether that is available or not?

Dr. Hourrigan:

I would doubt it.

Dr. Stamp:

We would, of course, want quantities of brain. We couldn't do it on one histological section. We would have to examine the brain right through, as you well understand from my paper yesterday.

Dr. Hourrigan:

Dr. Morris was describing a group of mice yesterday, and you were asking him about it, that I understand were inoculated with Cheviot material from one of the Cheviots I was just describing.

Dr. Stamp:

I asked a question of Dr. Morris yesterday. The Cheviot strain in the mice did not in fact transmit a second time, and there was some question whether the agent had been isolated.

Dr. Mulhern:

I think this point on contact is vital, and it certainly would be well, without trying to prolong this discussion, to try to get both views expressed as to what evidence thay have briefly to back up their point of view. In other words, Mr. Parry says in his point that he does not think it is spread by contact and what evidence does he have to bear that point out, and have these gentlemen, since they say

that it does spread by contact, give their evidence? I think this would be extremely important to the evaluation committee having this in the record that they could evaluate.

Dr. Anderson:

I think that point is well taken. Although it may have been in the discussion the last 3 days, it would at least focus it down to one point now.

Dr. Gordon, would you care to speak to that?

Dr. Gordon:

I will say exactly what I said before, and that is, taking account of the field evidence of earlier workers, especially that of M'Fadyean and Stockman, together with the experimental evidence of Greig and Gordon described in my first paper, followed by the experience of Stamp and colleagues at Moredun, see their paper, and the spread by contact in mice described by Pattison, my second paper, it may be concluded that under certain circumstances scrapie can spread from infected to susceptible animals. In sheep in my experience, this has been seen only in animals at pasture, and its occurrence may sometimes pass unrecognized because of the long period of time that may elapse between exposure to infection and clinical manifestation.

While this may not be the most important method of spread of the disease, it cannot be disregarded.

Dr. Dickinson:

In answer to this specific question I cannot go over the whole ground again. My full views with one exception are stated in the discussion section of my Suffolk paper yesterday. I would refer the evaluation committee to the transcript of that because it is copy, and I think I was reading from my script at the time. It is a summary of our views of the build-up of evidence, with one exception, and I would like to take this point up now because it is of interest here, and I have been seeing and realizing many things this week. I have learned a lot, and one of the things I have just been learning since I wrote that table on the board over there, we have got to go home and look at some pathology again. I have not mentioned anything in any discussions in terms of data other than by suggesting that there is so little data on this point as to whether ram transmission on occasion occurs. Ram transmission I am meaning at a surprisingly high degree; that is surprising in terms of any purely genetic hypothesis.

I promised yesterday to come back to this point and put a table on the board which people could copy down and use for whatever conclusions they wish to draw from it. I don't know whether you can draw anything strongly from it, but it is there and it does include both Cheviots and Cheviot-Suffolk cross cases.

You will see that ram VII, at the top of the board, we bought because in one flock he had left a lot of progeny affected, a surprising number. He was then handed on to another flock where we know he left eight out of eight affected progeny. He himself was unaffected until death at 6 1/2 years. This is the one that died with acute arthritis, or was killed because of it.

We bought this ram and tried to use him for specific experiments to attempt to determine whether his semen anyway transmits scrapie nongenetically to his progeny.

Now these experiments were reasonably well designed, but in the era before contact transmission of scrapie became very real to us. So the fact that we now have to admit contagion at an unknown level completely rules out these experiments based on this ram as being interpretable as due to ram transmission. They may be or they must be contagious evidence, but I would point you to the fact that this ram is different in terms of the behavior of his progeny when mated with similar groups of ewes from the progeny scrapie incidence of contemporary ram matings. Their progeny have the lowest incidence of scrapie, but the groups are badly balanced and it is difficult to devise any satisfactory test, but the clear impression from this data is that this one ram is most damaging.

I think this kind of ram is rare in occurrence, but he does occur; this is not a suggestion, a deduction from that data. And you will see over there in our experience when mated with "OF" unaffected Suffolk ewes half the progeny were affected with scrapie, a high incidence. When mated with Suffolk "OS" affected, the progeny--this is unusual because

he is leaving scrapie around--only three out of seven got the disease. When he is mated with "OS" unaffected we only have four there, half went down.

But when he is mated with cast Cheviot ewes--cast Cheviot ewes are ones having lost teeth due to age. They are old. We get them. So it means that scrapie, if it occurred in Cheviots would mostly have occurred before that age and it occurs fairly rarely--I state this on the basis of the fact that there are a good number of large institutional flocks of well recorded Cheviots in Britain and in our information indicates that scrapie does occur, but at a very low incidence, shall we say one or two percent. All I say is Cheviots are extremely unlikely to be related even in their fairly remote pedigrees to a case of scrapie. This mating group left three affected cases out of 19 progeny--these are Suffolk-Cheviot cross lambs, and it is that three there that I think the people this afternoon should bear in mind. They have got to decide whether it was another of those cases where we were infinitely unlucky in our choice of Cheviot ewes. They have got to decide whether it is relevant evidence.

But we have been unlucky so many times that my impression, no more, is that those three may support the rest of our understanding, loosely controlled as it is, of this ram as being a menace, and a menace which we cannot explain. But such rams do seem to exist, and I think Dr. Hourrigan might be having some information later on this point.

Mr. Draper:

I want to make one or two remarks complementary to those of Dr. Dickinson and also to reiterate one thing that Mr. Parry said. I am afraid it is a long while now--at least it seems a long while ago. He did say in the detailed analysis of the large table we presented that this depended rather heavily on this ram which appears never to have affected progeny. I just want to reiterate that remark. When people look at the analysis of that table this has obviously got to be taken into account, that it does depend rather critically on that animal. I think we did say in the original paper that there was only one animal of this type.

Secondly, my remarks tie up with the ones Dr. Dickinson has just made, that he has this animal which he considers very dangerous. Mr. Parry has this animal which appears to be the opposite. This is to say the least very interesting.

Dr. Stuart:

It wasn't clear to me at what stage of the game this ram showed symptoms of scrapie.

Dr. Dickinson:

He didn't. Never. He was killed at 6 1/2 years old. He had no lesions suggestive of scrapie in his brain.

Dr. Stamp:

I might say that to fit our views of the time we were very anxious that this ram should develop scrapie, and we did everything we could to make it positive. We looked at it morning, noon and night, and we imagined it had every sign of scrapie we could think of. But in the end it let us down. It did not have scrapie. We set out at the beginning of our experiments quite definitely with the idea that scrapie was genetically determined, a recessive gene, and the evidence has made us change our views.

Dr. Stuart:

Well, wasn't it originally stated in the early literature that scrapie was thought to have skipped generations?

Dr. Stamp:

Yes.

Dr. Jensen:

Dr. Stamp, has this potentially dangerous animal been studied as a possible excreter of virus of scrapie?

Dr. Stamp:

Again it was very difficult because remember this was in the pre-mouse era.

Dr. Jenson:

You don't have him now?

Dr. Stamp:

No, he is dead. Died some time ago. I think it is true to say we injected semen. I am pretty sure we injected semen.

Dr. Dickinson:

No, I attempted to collect semen. I was pretty good at collecting semen from rams, but this boy laughed at me.

Dr. Stamp:

Well, we did mean to inject semen.

Mr. Parry:

Have you brain blocks in formalin?

Dr. Stamp:

I should imagine so.

I am sorry, Dr. Jensen, you mean did we put the brain when the ram died into sheep. The answer is very definitely yes, and nothing happened.

Mr. Parry:

I think this was Dr. Jensen's point.

Mr. Yoke:

For those of us who are laymen, myself for instance, I think it should be pointed out that all this proves is that this ram had scrapie offspring in each one of these groups that the variation in numbers here is so small that they don't mean a darned thing.

Dr. Dickinson:

No, I simply attempted an experimental demonstration. It is a cause for worry, no more.

Dr. Anderson:

Mr. Parry, would you at this point comment on Dr. Mulhern's question, the basis for your opinion as to the effect of spread from contact?

Mr. Parry:

Yes. Firstly, I will make it clear that my evidence is entirely with the natural disease. I will point out that some of Dr. Gordon's evidence, as I understand it, was concerned with the artificial disease under experimental conditions.

Now my evidence was shown in a series of slides yesterday about introduction of rams into clear flocks, the intermingling of one breed and another, in fact, two breeds, one of which Dr. Gordon says is resistant to challenge inoculation. The introduction into a high incidence Suffolk flock of ewes that I thought were clear whites from another flock, and where they have been for 5 years and none has been affected, but only ten if I remember aright out of the 14 introduced are still there after 5 years. I think nine survived to 5 years. That is the gist of it. And so far as we can see at the present we can find no evidence; we have disclosed no evidence of spread by contagion under our natural conditions in those flocks in which the conditions are such that we might detect it.

You will appreciate that if you already have scrapie in the flocks we are quite unable to distinguish genetic spread from contact spread.

Dr. Anderson:

Mr. Parry and also Dr. Gordon, in your reference to spread by contact you rule out the passage of the agent through breeding as a means of contact?

Dr. Gordon:

I am afraid I cannot say whether the breeders of registered purebred British sheep have ever illegally introduced other breeds into their family lines and still registered them as purebred. Neither can I give an explanation as to how the disease passed from one purebreed to another, but at one time the disease was prevalent in Down breeds- in Hampshires and Wiltshires then, by some means, it spread to Scotland. How did it travel north; on a strain or in a gene? I cannot say how it traveled but it arrived all right and in the absence of your eradication programme I think the same would finally have occurred in America. Your present policy is probably eliminating all the susceptible sheep and their immediate progeny in flocks where the disease appears and by doing this you are reducing the distribution of susceptible sheep that may have been exposed to infection. I think, however, it will be clear in the minds of those who have to decide policy that they have a choice of two kinds. Either a control policy or an eradication policy. With a control policy, the disease remains notifiable so that its distribution in the country is known and slaughter might then be confined to the affected sheep and its relatives. In my opinion there is a risk here that unrelated susceptible sheep in other families might, if they have picked up infection, come down with the disease a long time

later. With an eradication policy in which the whole flocks and their immediate progeny are destroyed this risk is greatly reduced. It may be, however, that you are prepared to take this risk and if spread by contact to unrelated families occurs it would provide valuable evidence on this controversial issue.

Dr. Anderson:

On the conditions that exist as we believe they exist in this country would you recommend that type of--

Mr. Parry:

I will help you out, Dr. Gordon.

Dr. Gordon:

He will possibly help me out agreeing to a control policy, and this might, of course, be a compromise. It is not our function to recommend policy; we have to provide evidence and the members of the evaluation committee will assess this evidence and after due consideration they will recommend a particular policy.

Mr. Parry:

Dr. Anderson, if it would help, we should be very happy to make available to you semen samples from our proven white ram. I don't know whether he is going to survive another breeding season. As Mr. Steele said, we would be quite prepared to make available to you sons of this ram.

But we must point out that all we will say about Bleep, the proven white ram--he was born in the year of the first Sputnik--is that the sons of Bleep will not go down with scrapie if we are right, but their progeny may if your sheep stocks here carry what we would call the factor.

We would be happy to do either. All I would ask is that I might be permitted to have a close association and close control of anything that is done, not that I disbelieve you, but because then we should have the same observer error over here as happens at home.

I discount this sort of evidence because I don't know quite what to make of it.

Dr. Mulhern:

Is there any possibility that when you speak of the epidemics that have occurred in Britain over the years that your spread of disease was so widespread that this has accounted for resistance, because this is important to us in trying to evaluate where we are.

Mr. Parry:

Look, I am extremely diffident to use the evidence, but I will do so under pressure at your request. The history is that in Dorset and Wiltshire and the adjoining southern counties region that the then existing sheep stocks were largely eradicated, virtually wiped out about 1810 to 1820, wiped out and repopulated with different sheep stocks.

Now within my own experience there is an area of country between Cambridge and Bury St. Edmunds of about 30 or 40 miles by 20 miles wide which was largely populated with Suffolk flocks close folded on roots and arable crops in which scrapie became increasingly prevalent from 1940 onwards.

On my theory, knowing the rams which went into those flocks, this is perfectly explicable on the introduction of gray and black rams from source flocks. It doesn't mean to say it is the right explanation. They have, in fact, given up keeping folded Suffolk sheep. This is in part due to losses from scrapie. It is in part due to changes in general farm management and economics, and in many instances these have been repopulated with Scottish sheep. Now please let me stress that I personally would not draw any conclusions from this. I merely tell you that this is a fact as far as I know. I don't know what else I can quote to support your point.

The next point is that on our experience we should hate to have it notifiable. This in effect under British conditions would lead to suppression of a great deal of information.

Now may I just add one point--Dr. Gordon, I owe you an apology because I should have prefaced my remarks about evidence of spread by saying I was unhappy to accept the evidence in the literature, the historical evidence, as unequivocal evidence one way or the other. I don't know how to assess it. I doubt whether anybody can at this late stage. I prefer to leave it on one side and not draw any firm conclusions from it one way or the other.

There is just one point I would like to make. The Australian Merino sheep industry is based on an importation by Captain MacArthur of Camden Park, New South Wales, made from

George III's Chichester flock between about 1808 and 1810. I was in Australia for 3 years and I believe this to be true--they have never known scrapie until a recent British importation. The Merino sheep will take scrapie. This is quite evident from the reports in the 19th century. At that time around Chichester from the records, scrapie was severe in the whole of these southern countries. This doesn't mean the royal flock of Merinos did come in touch with the contagious agent but it might have done.

The other point is that the sheep stocks of North America were derived from European sheep stocks over, shall we say, the first 60, 70 years of the last century--forgive me, Dr. Carter, but roughly that. It seems to me stretching matters a little bit to suggest that an infectious agent was not brought too. It is, however, possible that it wasn't brought or that resistant stocks were brought by chance.

Dr. Stamp I think may have some--

Dr. Stamp:

May I just ask one question and I will make a comment, a rather facetious one. I must say I considered I thought pretty big in terms of hundreds of experimental sheep but I have nothing on Dr. Gordon and Mr. Parry; they want the whole of the sheep population of the North American continent to go at for their experiments. I haven't ever thought that big.

My question is whether or not it is wrong to go back to showing these sorts of anecdotes about what happened in the past.

Mr. Parry:

Which anecdotes?

Dr. Stamp:

About Suffolk sheep, and so forth and about certain areas being cleared. There is no proof of this.

Mr. Parry:

What I quoted from the Eastern Counties of England is from my own experience.

Dr. Stamp:

Yes, but you quoted from the 1820's. You had no experience in the--

Mr. Parry:

No, no, I said 1940.

Dr. Stamp:

No, but you went back to the 1820's.

Mr. Parry:

Oh, I am quoting Arthur Young.

Dr. Stamp:

Yes, well, these are just anecdotes virtually.

Mr. Parry:

Well, I prefaced my remarks with this.

Dr. Stamp:

Well, let's forget it. For heavens' sake, let's stick to scientific evidence.

The point I would like to ask Mr. Parry--I don't know whether he is prepared to answer this--but have you in fact evidence in your experimental flock, quite apart from your field data, that contagion may have occurred?

Mr. Parry:

May have occurred--I suspect it may have occurred. I said so, I hope. Did I not?

Dr. Dickinson:

Yes.

Dr. Stamp:

I just wanted to be sure.

Mr. Parry:

Oh, fair enough. In my experimental flock--

Dr. Stamp:

You are pretty certain contagion has occurred?

Mr. Parry:

Oh, no, I am not certain because I don't know--I now realize I don't know enough about the genotypes of the ewes I introduced.

Dr. Stamp:

You feel it might have?

Mr. Parry:

I feel it might have occurred, and what I have heard from you this week makes me think that I am probably in this spot.

Dr. Stamp:

Thank you.

Mr. Parry:

Yes, but I have got the artificial disease muddled up with the natural disease.

Dr. Stamp:

Well, this is a question of opinion again, this difference between the artificial and natural disease.

Mr. Parry:

No, it is not a matter of opinion. It is a matter of fact. You see, I have sheep on--

Dr. Stamp:

No, I don't mean in this sense. I mean in the deductions of whether you accept the natural disease as being different from the artificial.

Mr. Parry:

I used the term artificial to mean inoculation disease. I am happy to use any term.

Dr. Stamp:

Let's leave it to the meeting to make up their minds.

Mr. Parry:

I said what I meant.

Dr. Anderson:

Well, thank you very much, Mr. Parry. I appreciate it very much.

Paper No. 45

SCRAPIE IN THE UNITED STATES AND THE SCRAPIE ERADICATION PROGRAM[1]

J. L. Hourrigan[2]

Scrapie is an insidious disease of sheep and goats caused by a transmissible agent, characterized by a long incubation period and a progressive degeneration of the central nervous system which causes the affected animals to rub and scratch, become debilitated and incoordinated, and terminates in death in nearly all cases.

The disease has been known in Great Britain and countries of Western Europe for more than 200 years.

SCRAPIE CONTROL AND ERADICATION PROGRAMS IN VARIOUS COUNTRIES

Countries in Which Scrapie Has Been Reported

Although the history of scrapie, its origin and epidemiology, is distorted by hearsay and conjecture, early workers expressed considerable interest in the disease and it has been reported in Australia, Austria, Canada, England, France, Germany, Hungary, Iceland (where a similar disease is called Rida), India, New Zealand, Norway, Poland (Prussia), Scotland, Spain, Wales, and the United States.

Eradication Efforts in Australia, Canada, New Zealand, and the United States

In Australia and New Zealand scrapie was first reported in 1952 and is believed to have been newly introduced by imported British sheep. Eradication programs including quarantine and slaughter procedures were immediately instituted. In New Zealand, a second outbreak, apparently an extension of the previous one, occurred in 1954. Officials in both countries consider that their efforts to eradicate scrapie have been successful, and that the disease no longer exists in either country.

[1]Prior to publishing, the data presented was updated to June 30, 1964, in order that it would be more nearly current.

[2]Senior Staff Veterinarnian, Diseases of Sheep and Goats and Ectoparasites, Animal Disease Eradication Division, Agricultural Research Service, U.S. Department of Agriculture, Hyattsville, Md.

Scrapie was first reported in North America in 1939 in Canada. The first case reported in the United States occurred in Michigan in 1947. Both outbreaks are believed to have been associated with the importation of British sheep. Regulatory officials in the United States have worked closely with those in Canada as scrapie problems in the two countries are similar and are closely associated. The two countries have very similar Scrapie Eradication Programs.

Control of Scrapie in Other Countries

In certain countries where scrapie is endemic, owners attempt to reduce the incidence of the disease and the stigma of having an infected flock by slaughtering selected animals of certain bloodlines. These individual efforts do not represent organized eradication programs. Such measures are often helpful in suppressing, the disease but seldom eradicate scrapie.

LOSSES DUE TO SCRAPIE

Losses in Britain

During this seminar there has been considerable discussion concerning the incidence of scrapie in Britain and losses due to the disease. One of our collegues, in response to questioning, gave a tentative estimate of 5,000 to 20,000 cases of scrapie occurring in England per annum. These cases occurred in 23 million sheep, of which 11 million are breeding ewes. These figures were supplemented by an estimate of 400 cases occurring annually in the 20,000 Suffolk ewe population in England, or an incidence of about eight percent of the 5,000 or so replacement ewes added in England annually. Figures for losses occurring in five heavily infected flocks under supervision ranged from 11 percent to 35 percent of the ewe replacement crop becoming affected eventually. Field losses were said to range yearly from very low up to 50 percent in any given infected flock. The scientists from England and Scotland believe the estimates given are reasonable, but agreed that they were purely estimates because scrapie is not a reportable disease in Britain, and its occurrence is largely unreported.

We are given to understand that the disease in the 18th century was indeed a serious menace to many flocks. One can still read of the many statements made at that time in Britain, pointing out quite clearly that the disease was of common occurrence and caused substantial losses to flock owners.

We are told that scrapie losses may be in the order of 100,000 English pounds per year, plus an additional 100,000 pounds loss of export sales, or equivalent to $560,000 (United States) annually. Losses from scrapie often equal the total from all other diseases combined and thus double the annual mortality rate, which in scrapie-free lowland flocks is normally about five percent.

The incidence of the disease in any flock at the present time varies considerably. The number of cases of scrapie occurring in a year may be only one or two, as in commercial flocks selling lambs for slaughter, where scrapie not uncommonly occurs, especially in the purchased pedigreed animals. Since both the male and female lambs are sold off for slaughter, and many of the older animals are culled, or die of other causes before reaching the age of scrapie manifestation, the disease may cause limited losses in such flocks.

The flock incidence, that is, the number affected per 100 breeding females per annum, varies widely from flock to flock, even of the same breed, and from year to year. In a particular infected flock, an earlier speaker reported that 75 percent of the lamb crop eventually died of scrapie. It can be, and in some areas has been a decimating disease, forcing disposal of the remaining sheep in the flock.

One British worker indicated that many flocks may be considered free, that is, no manifest cases for at least 5 years; in many, the incidence is sporadic (1 percent) or low (1-3 percent); in some, it is medium (4-10 percent); and in a few flocks, it is high (10 percent, and may exceed 20 percent). In severely infected flocks, 25-40 percent of the second-crop ewes may die from the disease.

Another speaker reported that in 19 infected flocks studied, ewes had about a 10-percent chance of developing scrapie during

their lifetime (an average of about 4 1/2 years).

Losses in Other Countries

France--Estimates by different workers are 4, 4, 7, 15 to 25, and 10 to 30 percent of infected flocks.

Iceland--3 to 16 percent per year in infected flocks.

India--The incidence in 33 villages was given as 1 to 10 percent.

Indirect Losses from Scrapie

We are also given to understand that the heavy culling of scrapie families, disorganization of breeding programs, difficulty of becoming free again in a heavily infected flock, loss of repute, and resistance of buyers to purchase breeding sheep from infected flocks, all combine to make scrapie a dreaded disease. While some breeders manage to live with scrapie and, with varying success, to cull against it, others abandon the struggle and dispose of the flock (thus further disseminating the disease), often at a heavy financial loss.

Losses in the United States--Figure 1.

Since 1947, scrapie has been diagnosed in 138 flocks in 26 States--as shown on the map. Round-number estimates through June 30, 1964, indicate that 80,000 sheep have been slaughtered, with $2,100,000 Federal indemnity and $1,200,000 State indemnity, or a total of approximately $3,300,000 in indemnity payments to flock owners. It is impossible to determine the losses which may have occurred in the United States from scrapie had the Cooperative Scrapie Eradication Program not been initiated, because many of the animals destroyed under the program would have developed and disseminated scrapie. It is reasonable to believe the program has prevented the disease from becoming more widespread in the Suffolk and Cheviot breeds, and prevented spread to other breeds in the United States.

Figure 2 gives a breakdown, by fiscal year, of the 138 infected flocks, and 229 affected sheep. There were 6 Cheviot flocks--3 in Ohio, 2 in Illinois, and 1 in Oregon--and a total of 12 Cheviot sheep found to have the disease. The remainder of the known affected sheep, 217, were Suffolks. The affected sheep--63 rams and 166 ewes--were found in 106 counties in 26 States.

Figure 3 simply shows, in graphic form, the number of infected flocks and the number of States in which they were found. The highest number reported in any one year was 23--8 years ago in 1956. Since then a relatively lower number of from 7-14 infected flocks have been reported yearly.

Figure 4 includes all outbreaks reported up to October 1, 1958, and groups outbreaks according to probable origin. It is quite apparent that scrapie spreads as sheep are sold from infected or source flocks into other flocks, often across the country into distant States, or from one country to another.

Back-tracing from California outbreaks Nos. 1 and 2, where the eradication program began, takes one to a Canadian flock, since destroyed by Canadian officials, as a source flock. Further tracing from this possible common source also leads into Michigan, New York, Connecticut, and Georgia, Ohio and Tennessee, Virginia and West Virginia, Indiana and Illinois.

Indiana, in the center of the diagram, has had 23 infected flocks--more than any other State up to that time. The earliest case was reported in August 1954, and the most recent occurred in April 1959. Ten of these flocks, as well as two in Alabama, two in North Carolina, and one in Missouri, acquired the disease through movements of exposed sheep from one of the infected flocks in Indiana. These 15 outbreaks in four States resulted from the movement of 322 exposed sheep into 85 flocks in 14 States, a classic example of spread. Had the remaining exposed sheep not been slaughtered, additional outbreaks would undoubtedly have occurred. The two affected sheep in North Carolina were a 66-month-old ewe from the Indiana flock, and her 21-month-old, immediate progeny, the latter showing signs of the disease a year earlier than his dam.

I might add that notwithstanding the relatively large number of outbreaks in Indiana, it has now been more than 5 years since the most recent outbreak there. This suggests that in a

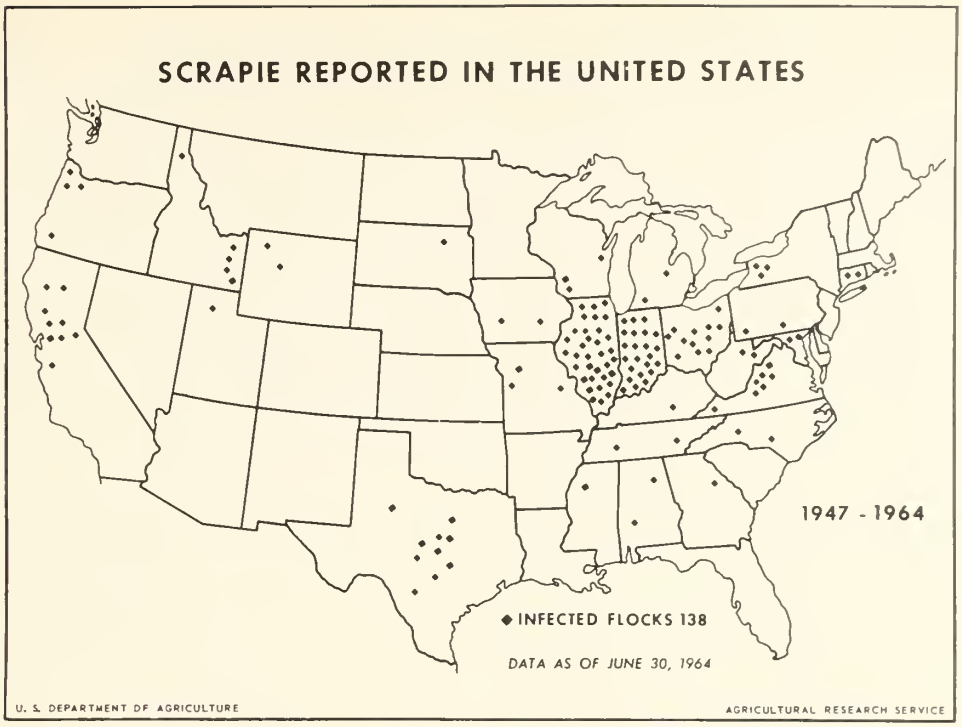

SCRAPIE REPORTED IN THE UNITED STATES
1947 - 1964
◆ INFECTED FLOCKS 138
DATA AS OF JUNE 30, 1964
U. S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE

Figure 1.

SCRAPIE REPORTED
1947-1964

FISCAL YEAR	AFFECTED FLOCKS			AFFECTED SHEEP			SEX		STATES	COUNTIES
	CHEVIOT	SUFFOLK	TOTAL	CHEVIOT	SUFFOLK	TOTAL	RAMS	EWES		
1947	0	1	1	0	9	9	0	9	1	1
1953	0	10	10	0	45	45	2	43	3	9
1954	0	3	3	0	6	6	1	5	2	3
1955	1	10	11	3	11	14	5	9	9	9
1956	0	22	22	0	36	36	14	22	9	18
1957	1	12	13	2	13	15	7	8	6	12
1958	1	6	7	4	6	10	5	5	7	8
1959	0	11	11	0	12	12	3	9	7	10
1960	0	11	11	0	12	12	5	7	7	10
1961	2	8	10	2	10	12	4	8	7	10
1962	1	13	14	1	27	28	8	20	4	12
1963	0	11	11	0	11	11	5	6	7	12
1964	0	14	14	0	17	17	4	13	3	11
1965										
1966										
1967										
1968										
TOTAL	6	132	138	12	217	229	63	166	26	106

DATA AS OF JUNE 30, 1964

U. S. DEPARTMENT OF AGRICULTURE

AGRICULTURAL RESEARCH SERVICE

Figure 2.

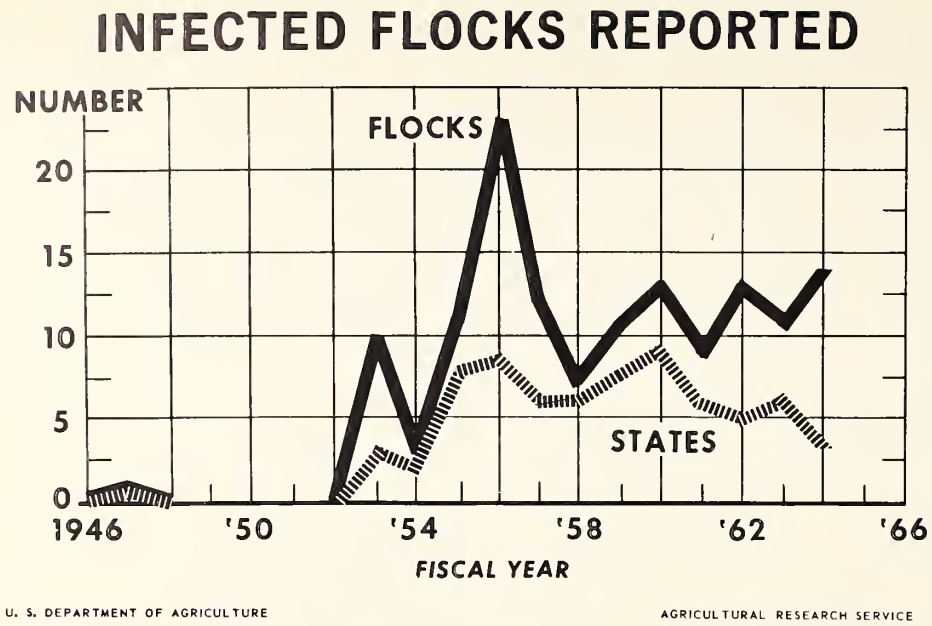


Figure 3.

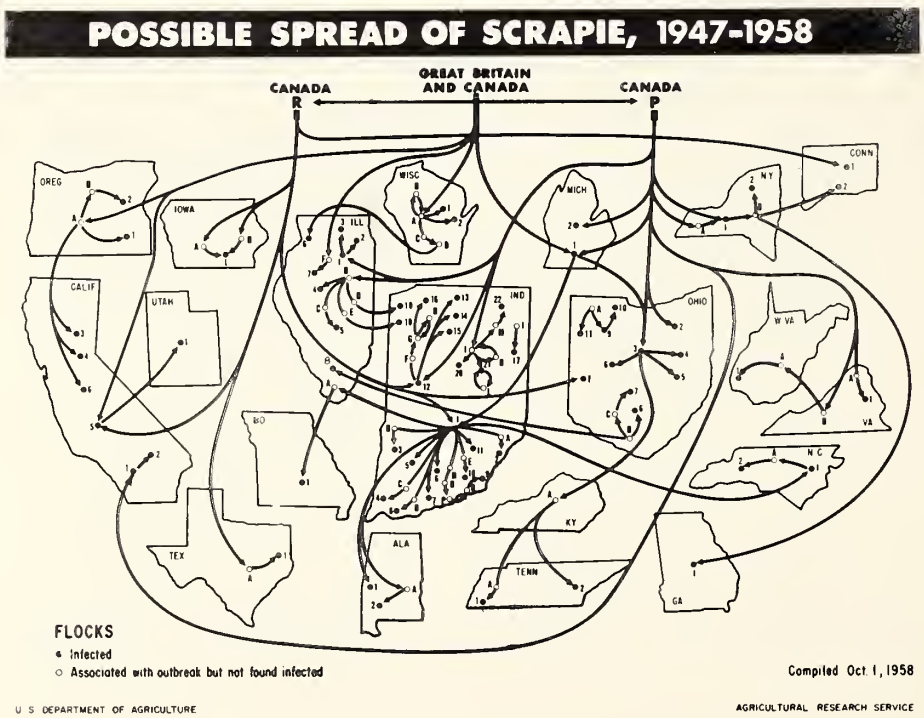


Figure 4.

State which has suffered 14 outbreaks in 1 year, the disease can be brought under control and, hopefully eradicated.

Figure 5 indicates the various means by which outbreaks of scrapie have been discovered.

Nearly half of the outbreaks were found through tracing movements of exposed sheep, and routine surveillance inspections. A substantial number were reported by veterinary practitioners. The owners themselves have often reported evidence of scrapie to regulatory officials or veterinary institutions and the disease has been brought to light through this means.

Four sheep with scrapie have been found at public stockyards where nearly 10 million sheep are inspected yearly by full-time, experienced, regulatory personnel. Currently, approximately 15 million sheep are inspected on farms each year, an important part of the Sheep Scabies Eradication Program. Two infected flocks have been found during these inspections. I believe these figures are impressive when one is thinking of the prevalence of scrapie in our general sheep population. Certainly the general incidence must be quite low or more cases would have been unearthed through these wide-scale inspections.

Although it is likely that sporadic cases can go undetected, we are of the belief that scrapie is not widespread in our sheep population, even among Suffolks and Cheviots, and probably does not exist in other breeds. We were not able to assess the possibility that there is present an appreciable number of subclinical cases--research has not been able to demonstrate that such occur--or whether carrier animals might play a significant role in the epidemiology of the disease.

Figure 6 shows the trend in the number of flocks under surveillance. Flocks from which scrapie-exposed sheep are removed are held under surveillance for a period of 42 months, and are inspected every 6 months. This is for the purpose of detecting the disease, should it appear in the flock. There are currently 731 flocks comprising 210,000 sheep in 30 States in this category. There were at one time, in 1956, 2,035 flocks under surveillance.

Figure 7 gives the comparative average price of rams sold at the National Ram Sale during the 14-year period 1950-1963. Some individuals had felt that the eradication program had adversely affected the price of Suffolk sheep, as the great majority of the scrapie outbreaks have involved this breed. In order to determine whether this had actually occurred, we obtained the selling price of the rams sold at the National Ram Sale, and compared the average price of Suffolk rams with seven other breeds, including Hampshire, Targhee, Rambouillet, Columbia, Suffolk-Hampshire cross, Rambouillet-Columbia cross, and Panama. These comparative prices indicate that the value of Suffolk rams has held up quite well; in fact, Suffolks have continued to command top prices, compared to other sheep.

Figure 8 reflects the age, in months, of scrapie-affected rams and ewes at the time of death, or destruction. These figures are similar to those given in earlier papers during the Seminar, and it shouldn't be necessary to dwell upon them.

On this graph the numbers of affected ewes and rams are expressed in percentages of the total of each, which gives a rather interesting comparison between rams and ewes. The curves up to about 50 months of age are quite similar. However, beyond that point the ewes carry on rather strongly to 80 months and even up to 112 months, or more than 9 years of age.

The rams, on the other hand, drop off abruptly near 50 months of age. One is inclined to suspect that this is because rams are culled, die of other causes, or are otherwise removed from risk earlier than ewes, and thus have less opportunity to exhibit the disease at older ages.

DEVELOPMENT OF THE COOPERATIVE STATE-FEDERAL SCRAPIE ERADICATION PROGRAM

The objectives of the Scrapie Eradication Program are prevention, control, and eradication of the disease.

Only two basic disease-eradication principles are applicable in attaining these goals. These are preventing exposure of susceptible animals, and/or increasing their resistance.

Research efforts to produce a vaccine to increase resistance have been discouraging. If either breed or individual animal resistance

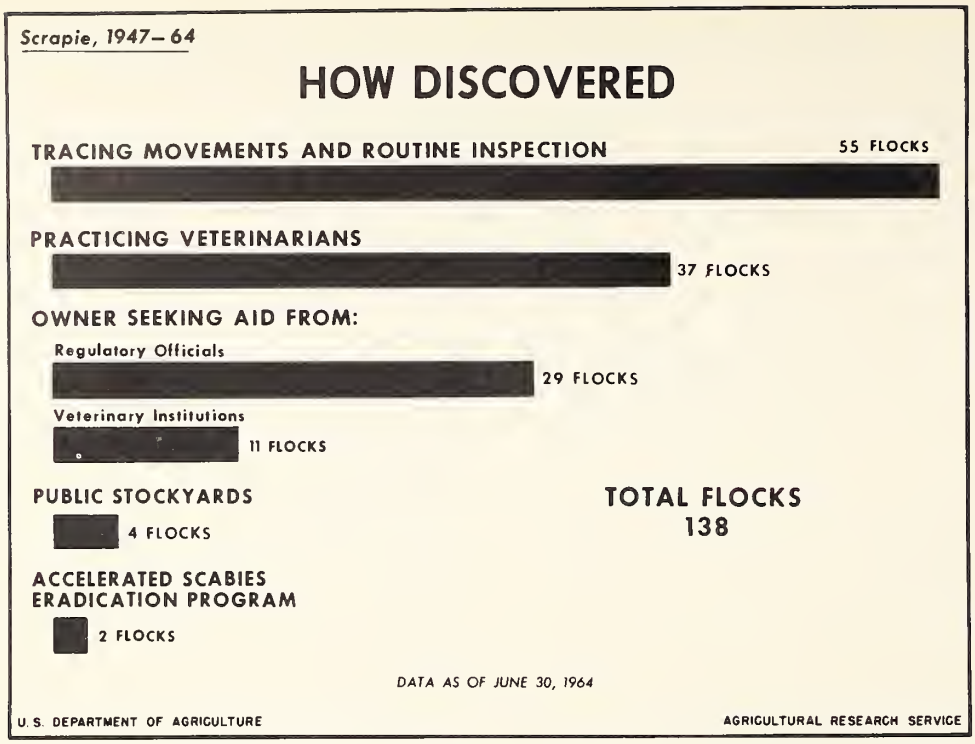


Figure 5.

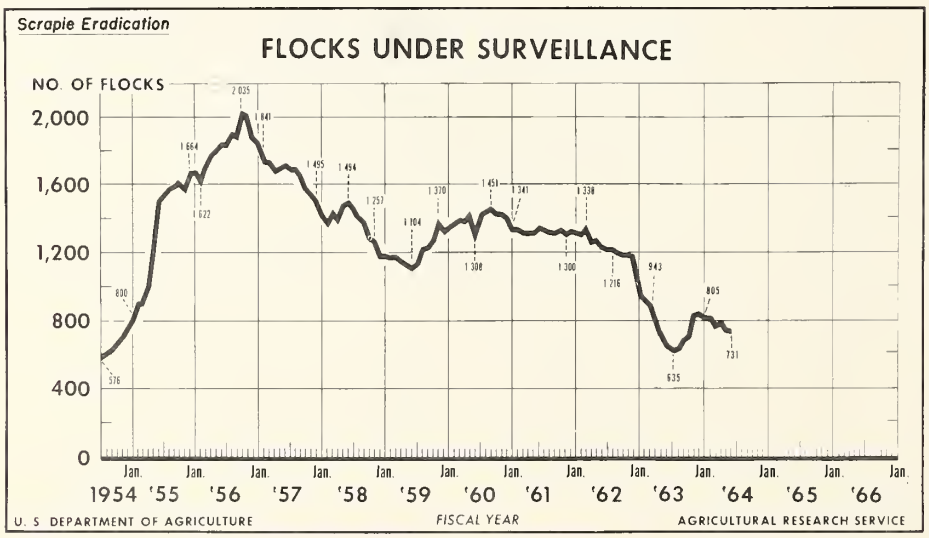


Figure 6.

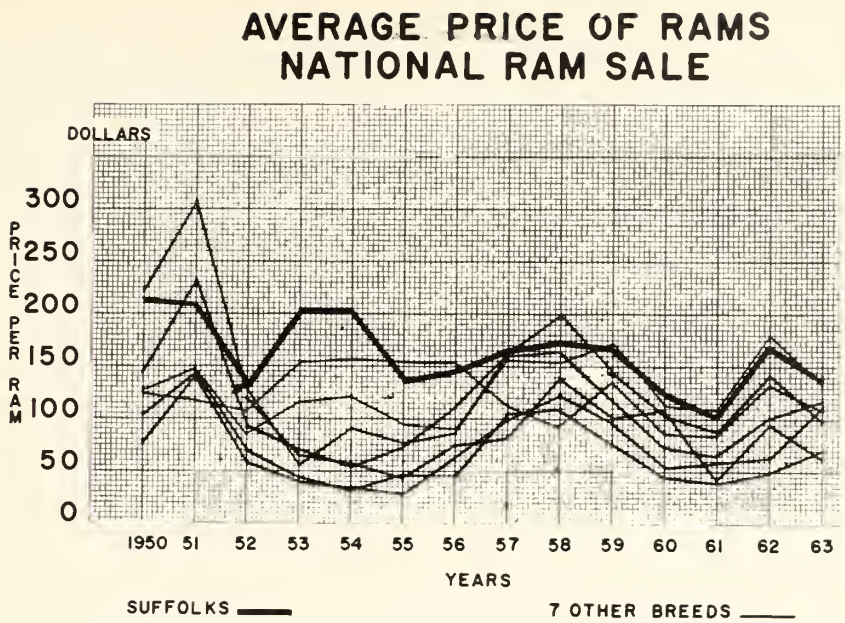


Figure 7.

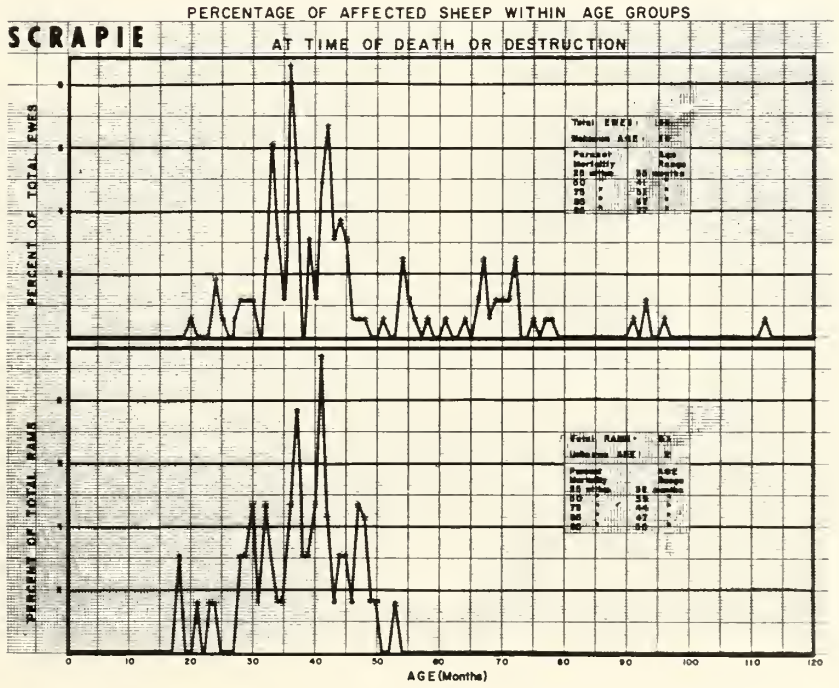

PERCENTAGE OF AFFECTED SHEEP WITHIN AGE GROUPS
SCRAPIE
AT TIME OF DEATH OR DESTRUCTION
PERCENT OF TOTAL EWES
PERCENT OF TOTAL RAMS
0 10 20 30 40 50 60 70 80 90 100 110 120
AGE (Months)

Figure 8.

occurs, it might be possible to develop strains of scrapie-resistant sheep. Even if possible, this would be a very long and laborious undertaking, and not necessarily compatible with breeding programs designed for other purposes.

Preventing exposure of susceptible sheep is the only presently known method of avoiding additional outbreaks. Research workers maintain that the disease may spread from parent to offspring, or by direct and indirect contact. Until research quantitates the relative danger from each, program direction must consider appropriately modified conventional methods which embrace both possibilities of spread. Since the scrapie agent has been demonstrated to be present in many tissues in clinically affected animals, it is clear that the visibly affected sheep must be destroyed. There is no diagnostic test for the disease in the live animal not showing signs; consequently, there is no way of knowing how many exposed animals may harbor the infectious agent. This factor, linked with the long incubation period, has necessitated the slaughter of exposed animals. The consensus has been that the immediate progeny of exposed animals may also be dangerous.

Following the first outbreak which occurred in Michigan in 1947, scrapie was again reported in October 1952, when, in a California outbreak, involving two associated flocks, 21 sheep were found to be showing signs of the disease. Shortly thereafter the California Department of Agriculture, the California Wool Growers Association, the National Wool Growers Association, and the United States Livestock Sanitary Association urged the Department of Agriculture to take immediate action to eradicate scrapie. On October 31, 1952, the Secretary of Agriculture, in accordance with Public Law 451, 82nd Congress, Second Session, and Section 11 of the Act of May 29, 1884, declared an emergency, thus providing the means for establishing the Scrapie Eradication Program. This emergency declaration was terminated on December 17, 1954, as provisions had been made to handle outbreaks without the declaration of an emergency. Like all animal-disease-eradication programs in the United States, it is a State-Federal cooperative program, with each agency having certain basic responsibilities.

Regulatory officials recognized scrapie as a particularly difficult disease to combat. I believe all of us here will agree on this point. It was essential that program procedures be both effective and practicable. In developing these procedures, industry representatives and research workers were consulted and all available literature and other information concerning scrapie carefully studied. On numerous occasions members of the industry, research workers, staff members of veterinary colleges, United States Livestock Sanitary Association representatives, other regulatory officials, and others met to discuss the scrapie problem and the eradication program. These meetings are well documented. In addition, representatives of these groups visited sheepmen and research institutions in Britain and France and consulted personally with authorities on the disease.

The program adopted in 1952 included laboratory confirmation of the disease in animals showing clinical signs of scrapie, quarantine and slaughter of all sheep and goats in the infected flock, and tracing and slaughter of all exposed animals moved from the flock and their immediate progeny. The 55 California premises involved were cleaned and disinfected. These procedures were quite effective and now more than 12 years have passed with no extension of infection from these outbreaks. California has since suffered seven additional outbreaks--not associated with those in 1952.

The disease was next reported in five Ohio flocks in 1953. The eradication program in Ohio included slaughter of infected flocks, but not exposed sheep moved from them or their immediate progeny. Instead, flocks including these exposed animals and their progeny were inspected. During these routine inspections, certain of the exposed animals and their progeny were later found to have scrapie in Ohio and Tennessee. During the same year, scrapie was diagnosed in three Illinois flocks. These flocks and exposed sheep moved from them were slaughtered.

Regulatory officials and others observed that scrapie was spread over great distances through the normal movements of sheep and that, in the absence of such movement, it did not seem to spread readily through an area. It became obvious that scrapie could be neither

effectively controlled nor eradicated unless source as well as infected flocks were properly handled, and that exposed sheep moved from flocks in both categories and their immediate progeny should be slaughtered also. Source flocks were defined as those determined, on ample evidence and after careful consultation, to be disseminating scrapie but in which animals showing signs of the disease had not been demonstrated. The eradication program was so broadened in April 1957. Some States had found it necessary to take this action earlier and had done so.

Indemnities for slaughtered animals have been paid in accordance with State and Federal regulations. Federal indemnity was increased in 1954. It does not exceed 50 percent of the difference between appraised and net salvage value of each animal and is limited to $25 a head for grade, and $75 a head for purebred animals. The amount of State indemnity paid is governed by State policy and regulation.

EVALUATION AND EFFECTIVENESS OF THE PRESENT ERADICATION PROGRAM

The present program has obviously prevented scrapie from becoming more widespread in Suffolk and Cheviot sheep and apparently has been effective in preventing its spread into other breeds. The program has prevented the spread of scrapie to a large number of sheep in more flocks than would otherwise have been the case. It has also been effective in controlling the disease to a point where, with more technical information from research, eradication of scrapie may be attained.

The consensus is that the program is holding the disease well in check; however, it is difficult to predict that the present program will achieve total eradication based on the present knowledge of the disease. One can point to evidence of the program's success in California and Indiana, where the results have been quite encouraging.

REVIEW OF THE SCRAPIE ERADICATION PROGRAM

It was determined that it would be desirable to conduct a further review and study of the problem of scrapie in the United States, to appraise carefully the procedures followed in the present program and judge them on the basis of research developments, to give attentive considerations to all possible solutions and procedures, to compare and evaluate these, and to recommend those which will best serve the sheep industry and the Nation. This has been done before, on numerous occasions; however, we are particularly fortunate at this time in that the program review has been immediately preceded by the scientific Seminar on all aspects of the disease.

Scientists, in sharing their considerable knowledge and experience with us, have been very helpful from a control and eradication viewpoint. From this point of view I should like, if I may, to very briefly comment on some of the significant aspects of this information. First, there has been some question as to whether a transmissible agent is involved in scrapie. The proof as to whether such an agent causes scrapie has been clearly demonstrated by taking material from the affected animal, introducing it into a second animal, and reproducing the disease in the latter. The disease can be passed through an apparently unlimited number of serial passages. Secondly, research workers at several institutions have taken material from scrapie-affected sheep and produced the disease in animals of a different species--goats, mice, rats, and hamsters. This further disqualified the theory that the cause of scrapie is merely a simple autosomal recessive gene. Research findings presented at the Seminar demonstrate that contact transmission does occur in scrapie and that, although the transmissible agent is more resistant to heat and other abuses than many viruses, it performs generally as a filterable virus and should be considered as such. Research work presented further indicated that some manner of transmission takes place from parent to offspring, and that the frequency of such occurrence is probably greatly influenced by genetic natural susceptibility or resistance.

STUDIES OF OUTBREAKS IN THE UNITED STATES

Each outbreak is carefully studied in an attempt to establish the epidemiology of the disease. Particular attention is given to signs,

laboratory findings, age, sex, breed, history, and pedigree of each affected animal, movements from infected flocks, and the possible source of the outbreak.

During the nearly 12 years the eradication program has been in effect in this country, it has not been possible to identify the source of some outbreaks. However, the majority fall into a pattern and indicate spread from a limited number of common foci. Once these foci are identified and eliminated, they cease to further disseminate the disease.

We, of course, attempt to compare and correlate our field observations with information developed by research.

Breeds of Sheep Affected

There has been belief among some American sheep breeders that scrapie is a disease which is limited to certain breeds of sheep, and any discussion of scrapie quickly raises the question of sheep breeds. This thinking on the part of sheepmen is perhaps encouraged by our fortunate experience that scrapie has been diagnosed in only the Cheviot and Suffolk breeds in the United States, as shown in figure 9, and in these breeds and in one sheep each of the Hampshire and Southdown breeds in Canada. However, a review of the literature and information presented during the Seminar gives ample evidence that the disease has been known to wax and wane over the years in many different breeds of European sheep. It is also generally accepted that the disease was prevalent in many breeds in Great Britain during the middle of the 18th century, and is presently not limited to any particular breed in France, where British breeds are not generally represented.

The opinion that sheep of most if not all breeds are susceptible to scrapie to a varying degree is further supported by research in Britain where Gordon was able to produce scrapie in 23 of 24 purebreeds challenged artificially, and where natural cases have been seen in 16 or more pure breeds plus breed crosses and various breeds in France. Gordon found that the susceptibility of animals of the different breeds challenged has varied from 0 to 78 percent of the animals inoculated in the test lots used.

Figure 9 also indicates that of the 229 affected sheep in this country, 166 have been ewes and 63 rams. The reason for the difference is, I believe, shown in Figure 8, which suggests that a greater percentage of rams are removed from risk at an earlier age.

Figure 10 depicts the possible spread of scrapie in Illinois. We see the same picture as shown in figure 4, except in greater detail. Again we observe the wide dispersal of sheep, and of scrapie, from common sources or source flocks. In a number of instances the sheep found to be affected had passed through several flocks over a period of time.

The situation in California, figure 11, has been that most of their outbreaks have resulted from sheep moved into the State from source flocks located outside California. Until the program provided for the slaughter of source flocks, this type of situation was often seen, with the source flock disseminating more scrapie than the infected flock.

Clearly, if one removes sheep of the infected flock and does not similarly deal with the source flock, he would expect the buildup of a greater number of cases resulting from movements from the source flock.

Figure 12 illustrates a situation in which a flock is quite possibly disseminating scrapie although one can't quite say with complete confidence that this is the case. A group of sheep in Virginia had been under suspicion for some time. The bulk of this group was sold and taken to Texas, where the same situation seems to prevail. In most instances the identification of the source flock is more clear-cut than this. If the flock concerned is in fact the source of outbreaks, time should make this more obvious.

It is an accepted fact that the scrapie virus is widely disseminated in the affected animal and that scrapie can be produced in sheep, goats, and laboratory animals inoculated by various routes with different tissues from affected animals, or by feeding animals tissues taken from affected sheep, goats, or mice.

The matter of immediate concern is--how the disease spreads naturally from one sheep to another. Obviously, it does, or scrapie would have eliminated itself centuries ago--or, perhaps, would never have existed. The possibilities to be considered are that the disease spreads from one animal to another by contact,

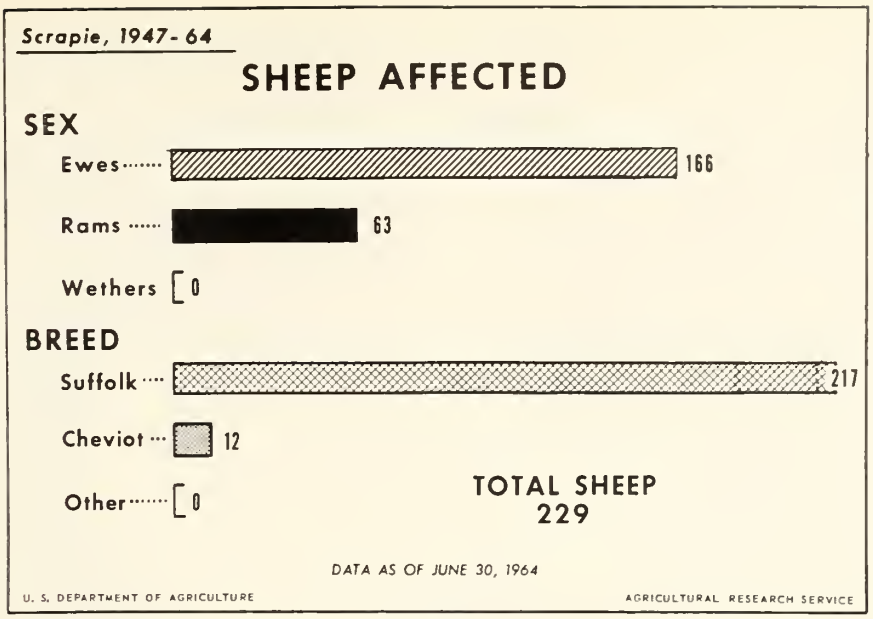


Figure 9.

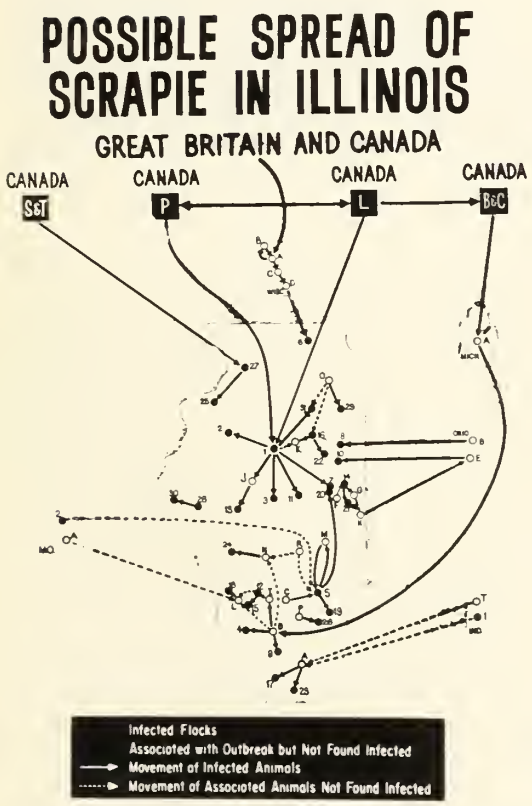


Figure 10.

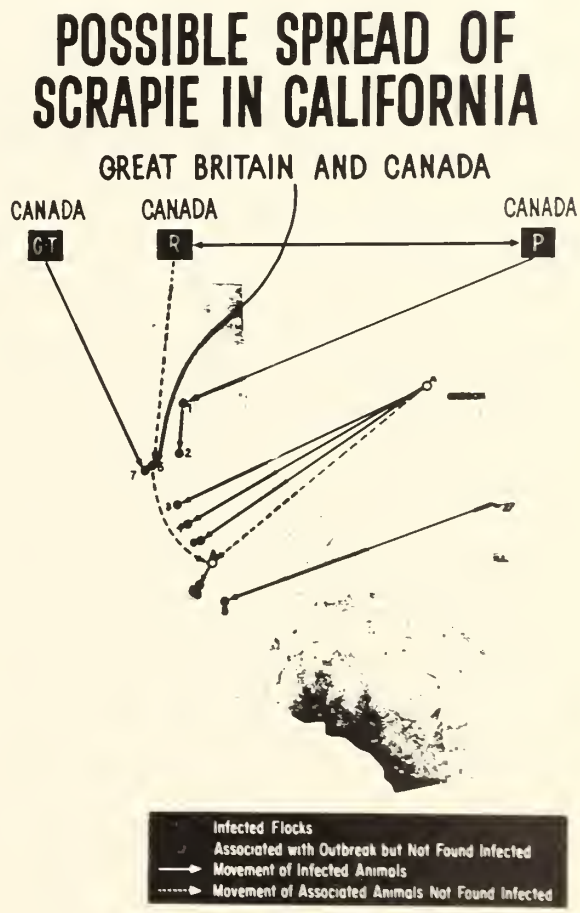


Figure 11.

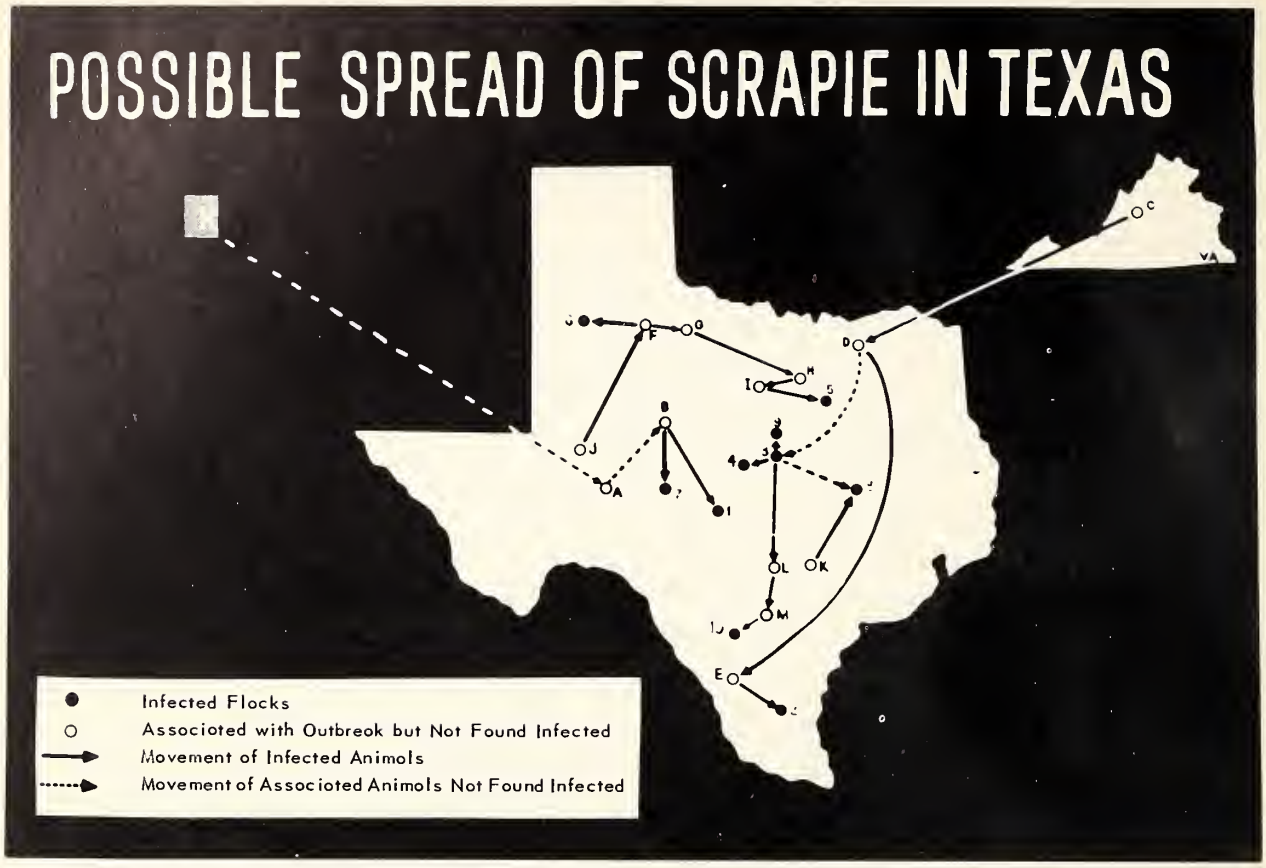

POSSIBLE SPREAD OF SCRAPIE IN TEXAS
VA
Infected Flocks
Associated with Outbreak but Not Found Infected
Movement of Infected Animals
Movement of Associated Animals Not Found Infected

Figure 12.

either directly through close association or by breeding, or indirectly through the medium of infected premises; or perhaps from the ram, or the ewe, or both, to their progeny. If the ram transmits the disease to his progeny, is the transmissible agent present in the semen? Is it in, or attached to, genes or chromosomes, using them as a means of transportation? In the case of the ewe, is the virus within the ovum? Is in utero transmission a factor? Does the lamb acquire the virus from the ewe's milk? Or does the intimate contact of ewe with lamb during early life provide the exposure? What are the genetic factors involved? These questions, by regulatory veterinarians, must in the end be answered by research workers. Such answers are not easily obtained by research work.

Importance of Transmission Along Bloodlines

Both experimental and field evidence suggest that scrapie can spread from the dam, and perhaps the sire, to the progeny. Many breeders who have had experience with the disease believe this to be true. Research workers reported during the Seminar that when two affected sheep are mated their progeny nearly always develop the disease. A much higher proportion of the progeny of affected ewes develop scrapie than those of affected rams. Stamp and Dickinson have reported that when maintained in a similar contaminated environment, 67 percent of the progeny of scrapie-affected ewes developed scrapie by $4\frac{1}{2}$ years of age and only five percent of the progeny of scrapie-free ewes did so. In this country, the pedigrees of affected sheep are carefully studied and bloodlines traced. The frequently recurring relationships observed in these studies seem too striking to be entirely coincidental. One must keep in mind, however, that similar apparent relationships might be observed on the basis of genetically acquired susceptibility or resistance with the susceptible animals being in an environment in which

they are exposed to the pathogen. Based on knowledge, presented during the Seminar, the latter seems to be a valid assumption. Some uninoculated offspring (both sheep and mice) of inoculated parents developed the disease. These, however, were also maintained in a contaminated environment.

Importance of Contact or Lateral Transmission

Although the theory that scrapie is spread from parent to offspring has many advocates, it does not fully explain the spread in certain flocks. As Gordon has pointed out, neither does it elucidate the means by which the disease spreads from one purebred breed to another. Examples are the two Canadian flocks in which a Hampshire and a Southdown sheep, as well as the Suffolks in these flocks, developed scrapie; and in Wales, where the disease appeared in purebred Welsh Mountain sheep (a breed believed free of the disease), following use of a Suffolk ram on a commercial portion of the flock. Animals other than his progeny came down with the disease. There may, of course, be instances of certain pure breeds being crossed with others; however, the characteristic appearance or performance of some breeds make their crossbreeding unlikely. There is experimental evidence that contact spread occurs in sheep, goats, and mice. The evidence is quite clear; however, in numerous instances investigators have failed to demonstrate spread by contact. One, then, is inclined to believe that contact or lateral spread does occur in the field, but may be of less importance in the epidemiology of scrapie than vertical spread from sire or dam, with emphasis placed on the role of the latter.

Research studies at Moredun Institute indicate that a likely means of scrapie spread is from an affected parent, particularly the dam, to lamb. Parry believes strongly that the affected ram is also responsible for scrapie affecting the progeny. Our experience in this country is that when half sisters or half brothers are affected, the common denominator is usually the ram.

In listing all sheep which are known to have had scrapie in this country, and identifying their common sires and dams, we find that there were many more sires which had two or more affected progeny than ewes that did so. This may be understandable, at least from a statistical standpoint, as rams have many more immediate progeny than ewes--perhaps on the order of a 25-50 to one ratio.

Figure 13 lists 35 apparently normal (to the best of our knowledge) rams which each sired two or more sheep or a total of 116, which were found to have scrapie. One of them, a British ram with Code No. 1, is known to have had 18 affected ewe progeny in California and Illinois. The remaining 122 affected sheep, out of our total of 229 in this country, were by 122 different rams not known to have had scrapie. We estimated that these 122 rams had sired an additional 26,000 lambs which are not known to have had the disease. This estimate is on a basis of 50 progeny per ram per breeding year.

Referring again to papers presented during the Seminar, we can easily note that these data do not fit the research data; otherwise, a much larger number of the 26,000 progeny should have eventually developed scrapie. We wonder why they didn't, and submit that the reason is that, again based on the research findings, many more would have developed scrapie had not the eradication program eliminated them before they did so.

Note that two of the affected progeny were by a ram who was a possible scrapie suspect on the basis of his history. He wasn't considered as affected on this chart as he died without a diagnosis being made.

The 63 known affected rams in this country have sired an estimated 7,500 lambs, none of which are known to have had scrapie. Again, this is quite surprising, if an affected ram plays a significant genetic role in the transmission of scrapie, unless the eradication program has been quite useful in preventing these cases from occurring. Otherwise, the research figures suggest that perhaps 25 percent of the 7,500 progeny of these affected rams could well have developed the disease. Lastly--an important program point before leaving this chart--is that if one identifies all half brothers and half sisters of known affected sheep in this country, largely by way of the sire of affected animals, he has located approximately 50 percent of all affected animals. This is an important program item because to ignore such animals is to get into serious difficulty.

NORMAL RAMS SIRING MORE THAN ONE SCRAPIE AFFECTED PROGENY

NORMAL RAM CODE NO.	NUMBER AFFECTED PROGENY SIRED	NORMAL RAM CODE NO.	NUMBER AFFECTED PROGENY SIRED
1	E E E E E E E E E E E E E E E E E E	18	E E R R (T)
2	E R	19	E E R R (T)
3	R R	20	R R
4	E E E E E R	21	E E E
5	E R R	22	E E E R
6	E E E	23	E E
7	E E E E	24	E E R
8	E E R	25	E E E E R
9	E R	26	E E
10	R R	27	E R
11	R R	28	R R
12	E R	29	E E E
13	E R	30	E E
14	E E	31	E R
15	E E R R R R R	32	E R
16	E E E E E	33	E E
17	E E E	34	E E
		35	R R

T = TWINS

35 NORMAL RAMS SIRED 116 AFFECTED PROGENY (9 CANADIAN) *

122 NORMAL RAMS SIRED 122 SINGLE AFFECTED PROGENY AND PERHAPS AN ADDITIONAL 26,000 PROGENY NOT KNOWN TO HAVE HAD SCRAPIE.

1 SUSPECT RAM CONSIDERED NORMAL IN THIS CHART SIRED 2 AFFECTED PROGENY.

THE 63 AFFECTED RAMS IN THIS COUNTRY HAVE SIRED PERHAPS 7,500 PROGENY, NONE OF WHICH ARE KNOWN TO HAVE HAD SCRAPIE.

R = 37 RAM PROGENY E = 79 EWE PROGENY

Figure 13.

Figure 14 is similar to Figure 13 except that it applies to dams rather than sires of affected sheep.

Considering the total of 229 affected sheep, in only six instances did two affected sheep share the same dam. In two of the six instances the affected sheep were twins. In all other instances different sires were involved.

The remaining 217 affected sheep of the total of 229 were progeny out of 217 different ewes. It is estimated that these 217 ewes had some 1,000 progeny in addition to those found to have scrapie. None of the additional 1,000 progeny are known to have had the disease.

Now, of the 217 other dams, one was affected (confirmed) and three others were believed affected, but brain tissues were not taken for laboratory study. Actually, the 166 known affected ewes have lambed perhaps 325 progeny, only four of which are known to have had scrapie; three of these were laboratory-confirmed. That doesn't say a greater number didn't have scrapie but if they did have it, we didn't know about it.

Now we have to take into account here--and I'm sure if I didn't say this, the statisticians would remind me of it--we don't really know how many of these were actually risks. Some of them were eliminated for various causes or died before they were old enough to show signs of the disease. Again, I think this speaks well for the eradication program because, if we are to accept the information brought to us here this week, it would certainly suggest that a considerably greater number remaining--perhaps as many as 67 percent of these 325 progeny of affected ewes--should have taken scrapie had they not been eliminated by the program.

Figure 15 shows affected progeny of 12 normal rams out of 33 different dams, and reflects the situations in California and in Illinois as seen during the early scrapie outbreaks.

In the two associated California flocks--considered one outbreak--20 ewes and one ram were found to have scrapie. Seven of

NORMAL EWES LAMBING MORE THAN ONE SCRAPIE AFFECTED PROGENY

NORMAL EWE CODE	NUMBER AFFECTED PROGENY LAMBED
A	E E
B	E R
C	E R TWIN
D	E E TWIN
E	E E
F	E R

6 EWES LAMBED 12 AFFECTED PROGENY.

9 EWE PROGENY
3 RAM PROGENY

213 NORMAL EWES LAMBED 213 SINGLE AFFECTED PROGENY AND PERHAPS AND ADDITIONAL 1,000 PROGENY NOT KNOWN TO HAVE HAD SCRAPIE.

4 AFFECTED EWES (3 NOT LABORATORY CONFIRMED) LAMBED 4 AFFECTED PROGENY.
THE 166 AFFECTED EWES IN THIS COUNTRY HAVE PERHAPS LAMBED 325 PROGENY--ONLY 4 OF WHICH ARE KNOWN TO HAVE HAD SCRAPIE.

Figure 14.

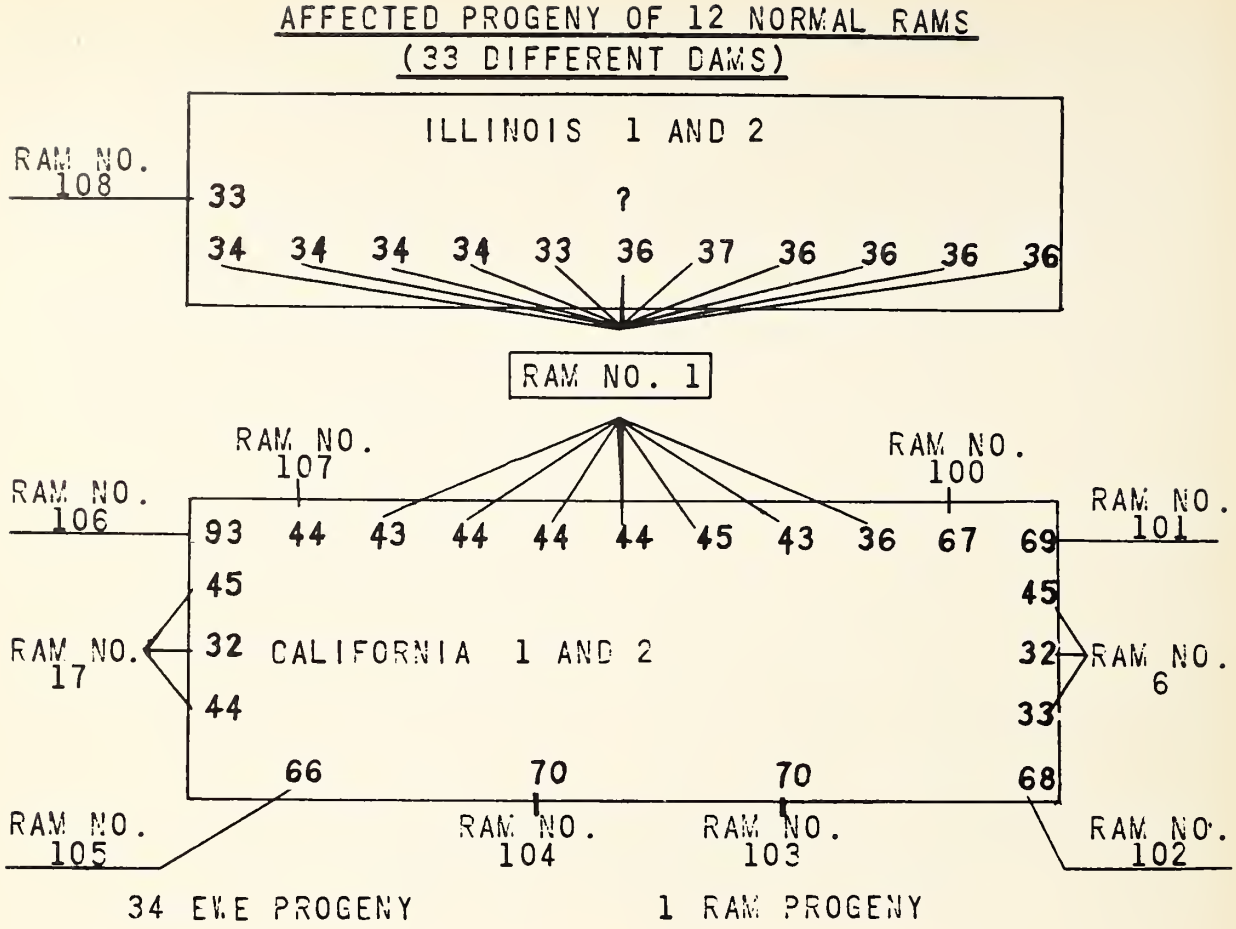


Figure 15.

them were the immediate progeny of British (imported into Canada) Ram No. 1. Their dams had been imported into California as bred ewes. Ram No. 6 sired three of the affected ewes, Ram No. 17 sired three, seven different rams sired one affected ewe each, and one ram, No. 107, sired the one affected ram.

These seven affected ewes sired by Ram No. 1 were from 36 to 45 months of age. Of the remaining 14 affected sheep, six were born on the premises and were 32-44 months of age. Eight had been purchased from other breeders. Two of the latter had been on the infected premises 24 months; the remaining six, four to five years. One of the eight purchased sheep was 44 months of age, the other seven were 66 to 93 months old.

It does seem significant that, with the exception of one, all the purchased sheep were considerably older than those born on the premises and all but two of the purchased sheep had been on the infected premises from four to five years.

All 21 affected sheep were out of different dams. One of these dams was considered affected but was not laboratory confirmed. If one assumes that the ewe plays a very important role in the appearance of scrapie in her immediate progeny, it does seem rather a rare coincidence that these 21 ewes--which were actually bred by 14 different breeders--should, at this one time and one place and each separately and in her own right, give simultaneous evidence of this phenomena.

It would seem that a more realistic hypothesis might be that a pathogen was involved, and the premises contaminated with this pathogen. One must also note that the extended pedigrees of these affected sheep, and I am sure other sheep on the premises as well, included

ancestors that one would be dubious about. This is also true of many sheep of this breed in this country.

In the Illinois outbreak--actually two flocks--the same ram, Ram No. 1, sired all except two of the affected sheep, and those two were, in fact, purchased out of the same Canadian flock where Ram No. 1 was located and eventually died. All of the 12 affected sheep had been purchased out of this flock and imported.

Again, the 13 affected sheep were out of 13 different ewes from four different breeders.

Figure 16, revealing affected progeny of four normal rams and eight different ewes, shows a somewhat similar picture on a smaller scale. Of the nine affected sheep, five were by Ram No. 16, while each of the remaining four were by different rams. Ram No. 16 had been sold to slaughter when 8 years of age. He had passed through at least three other flocks. All nine affected sheep were bred on the infected premises and were out of eight different, apparently normal ewes also bred on the premises. Two of the affected animals were out of ewe "E." This is one of the six instances in this country in which two affected sheep shared a common dam. One affected sheep, a ram, was 18 months of age; the others were 30-43 months old at the time of their death.

Figure 17, showing affected progeny of five normal rams and six different ewes, depicts a somewhat different picture. Six ewes in this flock were found to have scrapie. Two were by Ram No. 23 and each of the others was by a different ram. All affected ewes were out of different dams. The six dams were from five different breeders, and the five sires were from four different breeders.

Three of the six affected ewes had been bred in the infected flock, and were 24, 36, and 71 months of age. The other three had been purchased out of other flocks, and were 72, 72, and 75 months of age. The two for which records are available had been on the infected premises for 5 years.

There were some 1,700 sheep in this flock, with perhaps 500 in the scrapie age group. There were six known affected sheep, involving perhaps 10 different sheep families.

Figure 18 illustrates a series of outbreaks involving seven affected sheep in six flocks in four States. All seven affected sheep were

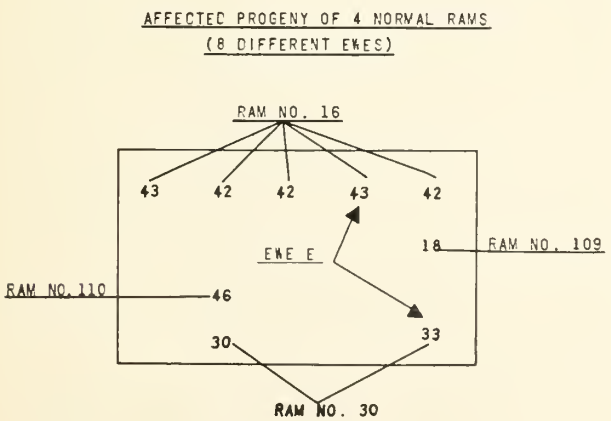


Figure 16.

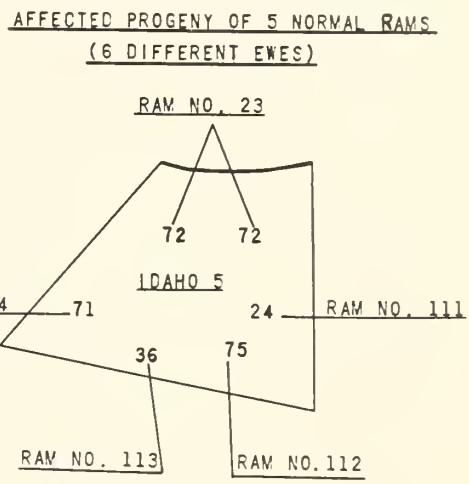


Figure 17.

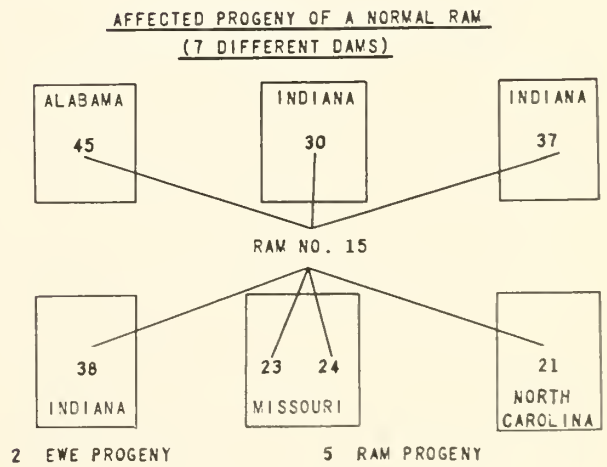


Figure 18.

by Ram No. 15, and were bred in the Indiana flock where Ram No. 15 was located. One affected animal was actually lambed on a North Carolina farm. Her dam, which had been purchased as a bred ewe, was also found to have the disease the following year--the only laboratory-confirmed instance in this country where both parent and lamb are known to have developed scrapie. One affected progeny of Ram No. 15 had remained in the flock of origin; the remaining five were showing signs of scrapie 6 to 30 months after being sold into other flocks. Two of the dams of the seven affected sheep had also been bred in the same flock as Ram No. 15; however, the other six dams were from six different breeders.

In summing up figures 14, 15, 16, and 17, if we assume in instances such as these that only vertical spread of scrapie occurs, we must then further assume that the individual affected sheep shown on these charts were destined to come down with scrapie wherever they might be. We must also assume that the numerous sheep families represented by these individual affected sheep were spreading the disease because of their particular bloodlines. This would mean that in this country we must be having many hundreds of cases of scrapie. Evidently this is not the case, and one must again turn to the hypothesis that a pathogen and a contaminated environment must also be involved.

Figure 19, the last for this paper, was prepared as a comparison of the relationships of each scrapie-affected sheep to each of the other affected sheep, to infected flocks, source flocks, and in relation to the sheep importations. The matters are all interrelated to such an extent that the graph is rather a complicated one. I do believe, however, that these comparisons bring out some quite valuable points useful in considering procedures designed to prevent further spread of scrapie.

It is important to keep in mind when studying this chart that the comparisons were made without taking any time elements into account. In other words, if four or five half-siblings by the same ram developed scrapie at about the same time in several different flocks, this fact was simply noted as such. The finding of one of the affected half-siblings in one of the flocks would not have enabled one to locate the others before scrapie appeared as the others were then also showing signs of the disease. When a particular relationship existed, this fact was noted and incorporated into the chart regardless of when or where or how these factors came about.

In the absence of information to the contrary, it was assumed that the sire or dam, or progeny of affected sheep or other sheep involved did not have scrapie. It is possible that certain of these animals may have had the disease and this fact went unnoticed.

Now, to give further study to the graph. We divided the comparative relationship into four graphs--strictly bloodline relationships of affected sheep, movements from infected flocks, movements from source flocks, and importations.

Bloodline relationships

In the bloodline group, we note that none of the 229 affected sheep were the first-generation progeny of affected rams (A), but that four were the sons or daughters of affected ewes (B), Two affected sheep were the second but not the first-generation progeny of affected rams (C), but none were grandsons or granddaughters of affected ewes. (D) Four affected sheep (two sets of twins) were full siblings (E). A total of 106 affected sheep, by far the outstanding numerical group, were found to be half-siblings via the ram (F). Thirty-five normal rams were involved. These were shown in detail in figure 13. Eight affected sheep were half-siblings via the dam (G) with details given in figure 14.

Thirty-four affected sheep were the second-, but not the first-generation, progeny of the sires of affected sheep (H), and six were the second-, but not the first-generation progeny of the dams of affected sheep (I).

Numbers indicated under F and G are most difficult to compare because, obviously, a ram has many more progeny at risk than a ewe.

Movements from infected flocks

Fifty-three affected sheep had spent some time in flocks found to be infected, (J), and of these, 30, or more than half, have been identified above in the bloodline group.

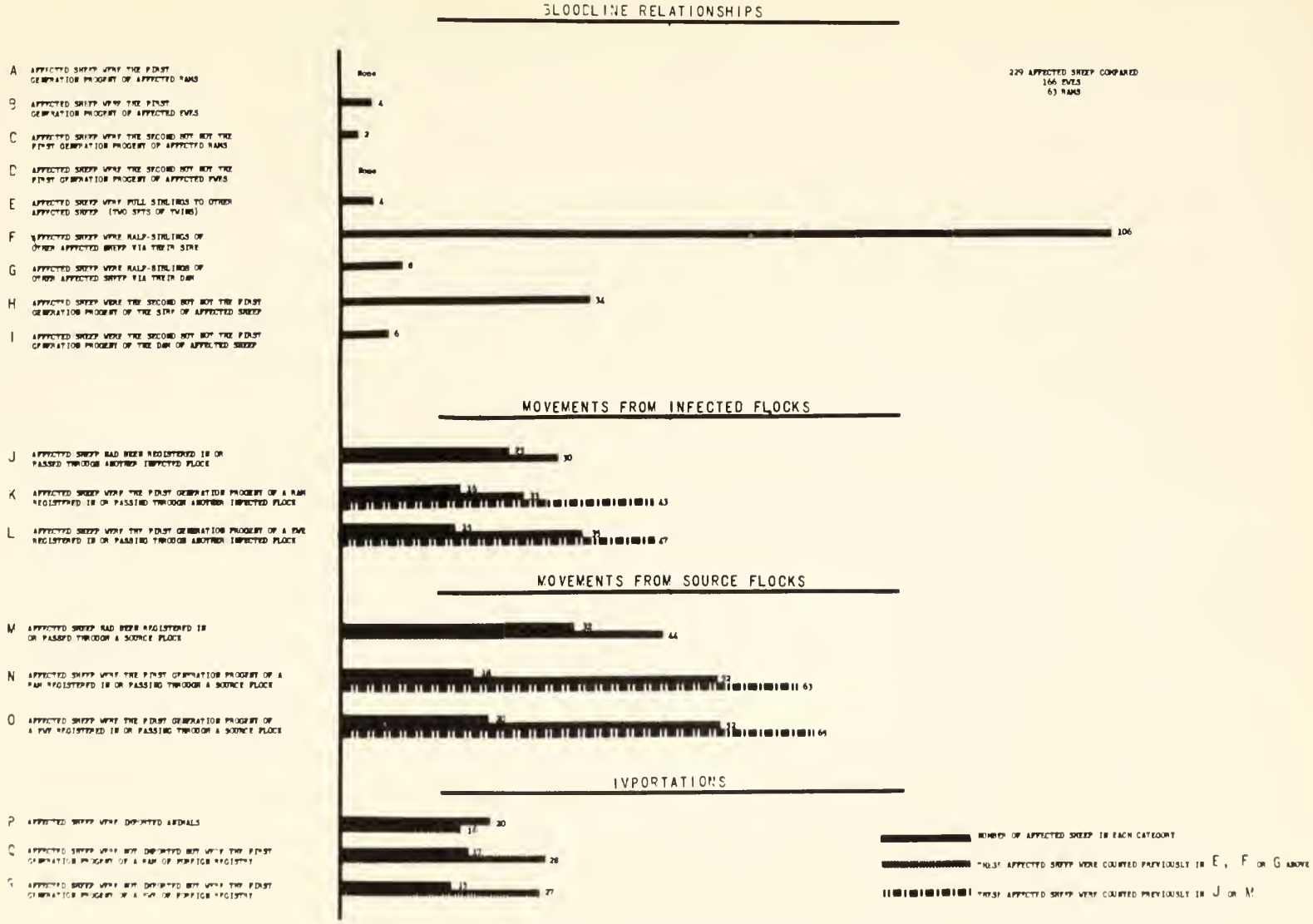


Figure 19. --A comparison of the relationship of individual affected sheep to other affected sheep, and to infected flocks, source flocks, and imported sheep.

Sixteen sheep were the first-generation progeny of rams which had some association with infected flocks (K). An additional number of affected sheep were also in this category, but were counted previously in E, F, G, or J.

(L) applies to immediate progeny of ewes of infected flocks. Approximately 50 percent of the 16 sheep (K) and the 15 sheep in (L) are duplicated as the affected sheep were both by a ram of an infected flock and out of a dam of an infected flock.

Movements from source flocks

M, N, and O are similar to J, K, and L except that source flocks rather than infected flocks were involved in the relationships.

Importations

P, Q, and R apply to affected sheep which had been imported or were not themselves imported but were the immediate progeny of rams and/or ewes of foreign registry--either Canadian, or British, or both.

One can get involved in various statistical traps in trying to interpret a complicated graph such as this one, and I don't wish to leave the impression that I am prepared to give it a final interpretation. Also, it was not possible to evaluate accurately all factors in every case. Complete records of Canadian and British sheep appearing in the pedigrees of affected sheep are not available. Imported sheep were reregistered in this country, and in some cases their previous identity as imported animals may have been lost. It is quite obvious, however, that if one puts his finger on all sheep having the bloodline relationships defined in A through I, he will have thus identified 164 or approximately 72 percent of the 229 sheep known to have had scrapie in this country.

Again, I am not prepared to say why or how that occurs, but nevertheless in our experience it did occur. Whether we are talking about scrapie as a genetic matter of susceptibility or resistance, or whether we are talking about maternal transmission, paternal transmission, or just contact spread, this has been the experience in this country.

The 138 outbreaks in the United States illustrate the potential danger of an insidious disease like scrapie in a country such as ours where lively trade among breeders can result in wide distribution of sheep and their diseases.

We look forward to additional research results, particularly those designed to explain natural transmission. Precise information in this regard will permit more exact identification of potentially dangerous animals and, it is hoped, provide for effective eradication procedures necessitating the slaughter of fewer sheep. This knowledge is especially important in the absence of a diagnostic test in the live animal.

We cannot look into a crystal ball and foretell what will happen during the next few years, but experience is convincing. The elimination of affected and exposed animals has effectively controlled the disease, prevented it from becoming widely established in Cheviot and Suffolk sheep, and has prevented spread into other breeds.

The chances for ultimate success appear good if we have an assurance of finding most affected animals and continue to follow sound eradication procedures and are supported by ample research.

SCRAPIE, ITS CONTROL AND ERADICATION IN CANADA

(Statement by K. F. Wells[1])

Mr. Chairman, ladies, and gentlemen:

First of all, I would like to compliment the United States Department of Agriculture on convening this meeting. We feel it has been most helpful to us with respect to our scrapie problem, and the scientific discussions have been extremely worthwhile.

I believe that there has been, possibly, a difference of opinion expressed on occasion; in fact it has been rather definite. I felt at one time that perhaps those expressing a difference of opinion were toying with intercourse on the basis of possible consummation and later marriage; but whether this is going to come about, or whether the marriage would be compatible, I am not exactly certain now.

But I think that perhaps the discussions here have brought two groups closer together.

My purpose in speaking now is merely to make comment. I am not going to draw any conclusions. I need not suggest that anything I say would have to be analyzed as evidence or fact; because I am not going to suggest that it is evidence or fact: it is, rather, opinion. If, in fact, anything is to be analyzed statistically or otherwise, I suggest that it should be my head for getting into this position in the first place.

However, the first thing that I want to do is to review briefly the Canadian scrapie picture.

Scrapie was first reported in Canada in 1938 by Schofield of the Ontario Veterinary College, and this was based upon clinical observations only on a group of 12 sheep, I think it was, imported from Britain into a flock in mid-Ontario. In the period of 3 1/2 years, three of the imported sheep showed clinical evidence of scrapie, and, during this same period, six of the imported sheep showed slight manifestations which one now presumably would call scrapie. However, according to our records, nothing, incidentally, was done about it, except that the sheep died. And according to our records, the owner, being able to afford it, grew tired of his hobby and destroyed the whole flock. That eliminated any evidence of scrapie insofar as that circumstance was concerned.

Now the second recording of scrapie in Canada was in 1942. On this occasion, a Cheviot ram, imported from Indiana, came down with scrapie, and was responsible for infection in four of the Cheviot flocks.

At this time, scrapie was made a reportable disease under the Animal Contagious Diseases Regulations, the infected flocks were slaughtered, and compensation was paid. Movements were traced, and where infection was found in the four flocks previously referred to, the flocks were slaughtered. Where no infection was found, the movements and the flocks in which they were established were kept under observation.

The next outbreak occurred in 1954, and since that time we have had 28 infected flocks, with 2 additional being identified as source flocks. This brings our total of infected flocks, officially diagnosed, or any that we have knowledge of, to 33, plus the two source flocks, which did not show any infection.

Since 1942, we have slaughtered approximately 13,600 sheep. We do not know how many of these sheep had scrapie, because our flocks were disposed of immediately upon the first diagnosis of scrapie in the flock. We did hold one flock at the laboratory for about 2 or 2 1/2 years, and nothing developed in that flock. These sheep have since been destroyed. They were simply held dormant at three of Dr. Plummer's laboratories, but nothing developed in them.

[1] Veterinary Director General, Health of Animals Branch, Department of Agriculture, Ottawa, Ontario, Canada.

But other than that we cannot tell you actually how many sheep had scrapie, or would have developed scrapie--and this is the conundrum, of course, that we all face--had the exposed or contact animals not been destroyed.

But we do know that all of the sheep on which every infected flock has been established go back in pedigree to four rams. In other words, eight cases out of our 33 infected flocks were the first progeny to one of our four pet rams. Fifteen cases out of the 33 were the second progeny to one of the four rams, or ram families; but in each case, these four rams.

So that this, unfortunately, is all we can say with respect to the number that might have had scrapie.

Now the Canadian policy, of course, is similar to that of the United States brought into effect in 1959. Infection in Canada has been established in four provinces only: Alberta, Ontario, Manitoba, and Nova Scotia. Now the Canadian policy at the moment is up for the usual annual review. So this meeting is for us rather fortuitous.

We have come to the meeting with our minds not completely made up in any way, but completely open. We have some people who assure us that their minds are made up and they don't wish to be confused with the facts. We are not quite in that position. And we have other people who, like a drunk man leaning against a lamp post, are using statistics for support and not for light. We try not to do this.

Now I don't want to become seriously involved in the scientific discussions which have taken place. In fact I don't intend to. But I believe I have to say what I do believe about scrapie.

First of all, I believe scrapie is a serious disease which might well, under certain circumstances, reach serious proportions.

Secondly, I believe that scrapie is a disease in which there is a transmissible agent as the causative factor.

Thirdly, I believe that the disease shows a propensity to follow in family lines. And I am not prepared to say why I believe that; whether it is because of the straightforward genetic theory, or whether it is because of the resistance or susceptibility theory, or whether it is because of the contact of offspring to dam which provides the family picture. But I do believe there is this propensity to follow family lines.

And fourthly, I believe that the disease can spread from sheep to sheep by contact, or from mouse to mouse by contact, or anyone of these combinations.

Now, having said what I believe about scrapie, I must now move into the field of comments on control of the disease. In starting these comments I must deal in some way with a sort of philosophy about scrapie. In fact, someone has said that scrapie in all probability is a philosophy rather than a disease.

These remarks obviously are based upon our interpretation of observations. I am not a scientist. I make no pretense at being involved in the scientific activities of our country which lead to research work. I am responsible for it, in the final analysis, but nevertheless, I don't pretend to carry it forward.

We take known scientific facts and mold these into a workable control effort. In many cases it is rather difficult to ascertain where science is, or what part science has played in the workable field effort that has been involved. But nevertheless these circumstances or field efforts work. As Dr. Hourrigan has said, we have successfully eradicated tuberculosis, brucellosis, hog cholera, foot-and-mouth disease, and a number of other diseases, utilizing scientific facts, and keeping field conditions in mind. But in dealing with these diseases, in all of them, we have been working with much fuller facts and scientific knowledge than we have with respect to scrapie.

In Canada, the general infection rate we believe is low. We don't know why, because we have been importing sheep from Britain for over 100 years; in fact, the basic stud of Canada--and, incidentally, of the United States--is, of course, British. Why, over this vast number of years, we haven't had more scrapie than has become evident, we do not know.

I think the same situation exists with respect to Australia, where again, the basic sheep population came from Britain. Australia has to my knowledge reported only one outbreak of scrapie in quarantined animals. These

were eradicated, and they have not had any more.

Now, if spread by contact is a fact--and I believe it to be--then my only opinion, or thought, with respect to this is that spread by contact in pasture depends to a great extent upon the density of the population. We have a very good example of this with respect to our rabies control.

We have in Canada a source of rabies in the wildlife, in foxes. And we know that the spread of rabies from foxes to our domestic livestock and dogs is dependent totally upon the density of the fox population. We follow the fox population closely in all parts of the country through our wildlife authorities. And we know that if our fox population is at the level of 1 or less per square mile in a particular area, we are not going to have rabies problems in such an area. We know as soon as the wildlife people tell us the fox population is getting up to 1-1/2 per square mile, that there will, in all probability, in that locality, be some problems with rabies, with foxes biting cattle, horses, dogs, sheep, and what-have-you.

We know that when the population gets up to two or three foxes per square mile we are, in fact, in so much trouble that we move men, materials, and equipment into the area in advance. We are that sure that rabies is coming as a result of this density in population.

Now, we must therefore conclude from this that densities certainly of what we call, with respect to rabies, the threshhold level must bear some relation to this spread by contact of scrapie. And I feel that this, then, can only be the logical explanation of why we have not had in Canada greater amounts of scrapie, and the same thing would apply to Australia--greater amounts: because the conditions under which we keep sheep are so vastly different from those in which the majority of the breeding flocks are maintained in Britain.

I must therefore conclude, while recognizing the fact of spread by contact, that this spread under our sheep-raising conditions is of low level, and might well be of little significance in field control; bearing in mind by "contact" I am not including contact of offspring to dam, and close confinement as in experimental work.

Secondly, I must conclude--bearing in mind our experience with four families being involved in every outbreak--that family lines in Canada, all in purebred breeding flocks, is our most serious menace in the spread of this disease.

Thirdly, I must conclude--and I feel this might be one of the few points on which the scientific advisors here will agree--that there is no test to determine the existence of the disease in advance of clinical symptoms.

Fourthly, I must conclude that there is no known means of readily destroying the causative agent of scrapie. Again I must conclude that a farm premise once having had infected sheep on it cannot be satisfactorily cleaned and disinfected to eliminate any chance of reinfection when the premise is restocked with sheep. Based on this assumption, then, if one were to carry forward a true policy, it would mean that where infected sheep had been on premises that these premises would have to be for many, many years prohibited to be restocked with sheep.

Fifthly, and lastly, I am convinced and confident that with the vast amount of research work now being done by highly qualified workers, many of whom we have heard here, that we will, in due time, know the answers to many of the questions puzzling us today.

Now, let me briefly state my understanding of the general principles applying to eradication of an animal disease by slaughter--or to use the European term--the stamping-out method, or policy:

First, we feel it can only be justified if there is good prospect, or a reasonble prospect, of success.

Secondly, we feel that the costs involved, not only in money, but also in animals slaughtered must have some economic relation to the impact of the disease.

Thirdly, our eradication schemes are usually applied to diseases having a serious economic effect. But, of course, it can and should apply to a less economically serious disease if it has been newly introduced into the country and has not gained a broad-based foothold, such as we believe scrapie must have.

Fourthly, of course, for any eradication scheme in the past we certainly have required--and I think it should be one of the main principles for the scrapie program--a

diagnostic test in order that we can get ahead of the disease; or, in place of the test, a short but definite incubation period.

Now, we have successfully eradicated hog cholera on many, many occasions as it has been introduced throughout the years, without a diagnostic test. But, of course, as you are well aware, we have in hog cholera a very short and sharp incubation period which, in fact, permits us to get ahead of the disease. We feel that without this incubation period we could not be successful in eradicating the disease, which we are always working behind. In fact, in our more serious hog cholera outbreaks, we almost come to the point where we have to, because of pressures of staff and time, forget about dealing with immediate cases except to get them under the ground. Our main emphasis is on tracing and getting ahead of the disease, and then working back, rather than keeping behind it and trying to catch up with it; because this, believe me, is a difficult thing.

So I raise the question: Does scrapie, as we now understand it, fit into these requirements? If it doesn't fit into these requirements, these eradication principles--and I sometimes question if it does--where, then, does it fit?

Here, I think we might examine control with an eye on future eradication when additional knowledge and tests are available. I would hesitate to suggest to anyone, and certainly I would never suggest it to my Minister, that we or any other country, give up adequate control of scrapie until (if and when--and I assume that we will get more knowledge of the disease) we have the diagnostic tools or weapons that are necessary to make control economically sound. And I would hope that we, in our country certainly, will move ahead and eradicate this disease, because I am convinced if this is not done, in the long run it could, and in all probability will, be of serious economic effect to our sheep industry.

In reflection then--and when I say "reflection" I really mean hindsight; and as you are aware, hindsight is a rare gift which is enjoyed and employed by practically everyone--but in reflection, having concluded that scrapie is a menace and could increase in intensity; the disease has a transmissible agent as a causative factor, and tends to disseminate through family lines; it can spread through contact, but under normal conditions in our country this method does not appear to be highly significant; that family lines in Canada certainly have to be involved, or certainly appear to have been involved in each outbreak--reflecting that we have no diagnostic tests available; that it is impossible to clean and disinfect a premise: I ask myself the following questions--merely ask myself, without answering them at this time, either to myself or to anyone else, first:

In Canada, with our present knowledge of scrapie with its long incubation period, no certainty as to the manner in which it is transmitted, no diagnostic test, and certainly no cleaning or disinfection means, can we eradicate the disease? Or are we, in fact, operating on the basis of a control policy?

Secondly, I ask myself: In view of the lack of real evidence covering field spread of the natural disease, are we justified in killing contact animals other than those classified as family progeny?

Third, in the light of evidence, I have to ask myself: In view of the impossibility of cleaning and disinfecting premises, what is the advantage of removing all sheep from an infected premise unless we are prepared to maintain that premise totally unstocked?

The fourth question I ask myself--and the last one--Have we, as a veterinary regulatory agency, been guilty of blindly applying the dogmatism of laboratory findings to a field eradication program without examining sufficiently carefully the total implications of our action.

Thank you.

DISCUSSION

Dr. Anderson:

Thank you very much, Dr. Wells. We look forward to your being with us the rest of the week, and sitting in on our meetings this afternoon and tomorrow.

I would like to recognize and introduce two gentlemen from Canada. They are breeders of sheep. Mr. Raymond Clarkson, President of the Canadian Sheep Breeder's Association,

and Mr. John Wilson, who is Vice President of that Association.

We certainly appreciate your participating in our discussions. And I would like to ask if you have any comments you would like to make at this time, or would you like to question any of the scientists who are present.

If not, we hope you have a safe journey home.

Dr. Dickinson, we will now give you the opportunity to comment on Dr. Hourrigan's presentation.

Dr. Dickinson:

I think what I have to say is important, because some members of the sheep industry have made great, and I believe wrong, play of some of the data here in the United States.

The evaluation panel--and it is to them that I am trying to address this situation (and I also wanted these Canadian friends to be here because I think it is an important fact to bear in mind, in view of the statements which were made by various people)--considered statements in the hearing before the House of Representatives' Subcommittee on this very question.

I am going to pick out one person who is, I think, in this audience, and invite that person to say whether he is willing still to stand by the statements which he made, and the interpretation of them for this particular purpose.

The person to whom I refer is Dr. Thomas L. King.

I would like to read the second paragraph in your presentation here.

> "In the ten years of the scrapie control program, only 200 of the nation's 30 million sheep have fallen ill with scrapie. Thus the incidence of scrapie is at the rate of one case per 1.5 million sheep."

Now I take it from that statement that you are wishing to suggest that, on the basis of the statistics which we have had presented here this afternoon, you could conclude that scrapie is not a particularly important disease to the sheep breeder.

Now could I invite you to confirm that that is still your conclusion; or are you wishing to withdraw this particular conclusion? Because I am going on, if you are not willing to withdraw it, to show how, on logical grounds, your conclusion is absolutely invalid.

Dr. King:

May I have your question again?

Dr. Dickinson:

My question to you is: By taking the number of cases observed in the United States and dividing them by the sheep population of the United States you have arrived at an incidence of the disease, in your mind, and from that very low incidence which you produced by dividing that one figure by another, you arrived at the conclusion that scrapie then is not important to the sheep breeder, his losses due to the disease are small--so small as to be insignificant.

Do you believe that this is an insignificant disease, or is it an important one which, without the presence of the United States Eradication Program in its present form, or possibly a modified form, would become to the sheep breeders here a very important disease?

Dr. King:

It is a significant disease. It is potentially an important disease.

Dr. Dickinson:

You are then withdrawing the statement.

I skipped two paragraphs down here in your statement. I was, in fact, quoting at you your own words. "And scrapie, then, is not important to the sheep breeder. His losses due to the disease are so small as to be insignificant."

You are withdrawing this view, and you have altered your mind on this issue, have you?

Dr. King:

Let me complete the answer which I began, and then I will take your new question.

It is not significant in the explosive sense. It is not a significant disease in the explosive sense of an epidemic, and that was the point that I wished to make in this statement.

Now as to the question of whether I have changed my mind, I must say at this point--and I expect to say more on this later--that I have, as I am sure many other people this

week have, learned a number of things which have altered my viewpoint to one extent or another.

Dr. Anderson:

Thank you very much, Dr. King.

Dr. Dickinson:

Could I ask for your additional comment?

Is it that scrapie is not an explosive disease--I think those were the words that you used: I think you see my sense--is it because, or is it possible that it is because, of the eradication program? I say, "Is it possible?" I'm not saying that it can be proved from the data at all. Is it possible that the program is being very effective, and this is why one only has a small number of cases? Or do you, as I would suggest have to say that from the United States data alone, taken in their own right--and only there--you can make no deductions one way or another; but only by inference from our own data can you conclude that the low incidence here--the entirely different pattern of its appearance as presented by Dr. Hourrigan this afternoon, is very clear evidence that the eradication program is sufficient to have caused the non-appearance of a massive number of cases of scrapie in the United States. Whether the program is efficient, or whether an equally efficient end-point would have been achieved by a modified program, is not something to which I am trying to address these remarks.

Dr. King:

I will give one more answer. And if there are further questions I would prefer to make a statement, which I am prepared to make, and then answer questions.

I have seen no evidence in the figures that have been available to me--and let me say that I cannot say I have seen all the data which the Department of Agriculture has; far from it--but on the data available to me I have not seen an indication that scrapie has been greatly reduced. Some, certainly.

The data given by Dr. Hourrigan were not in sufficient detail to give a complete evaluation on that point; nor do I feel capable of giving that kind of an evaluation.

Dr. Anderson:

Thank you very much, Dr. King. We will call on you later for discussion.

Do you have anything further, Dr. Dickinson?

Dr. Dickinson:

I think there is just one point.

I must say that I differ from this viewpoint. And I must say that these data are quite out of court for drawing any conclusions about the effectiveness of the United States Eradication Program, and the incidence of the disease, simply because one does not know the proportion of eradicated sheep which would have developed scrapie; and without that knowledge you cannot make any certain conclusions as to these questions.

Dr. Anderson:

I intend to call on anyone who wishes to comment on the eradication program, or to express their views. And at this time I will call on Dr. Thomas King from White House Station, New Jersey.

THE SCRAPIE PROBLEM IN THE UNITED STATES

(Statement by T. L. King[1])

On behalf of the American Cheviot Sheep Society I take this opportunity to commend the Department of Agriculture for arranging this seminar to provide the latest available scientific information on the nature and magnitude of the scrapie problem. Especially, I wish to thank our friends from across the sea for taking the time and the trouble to prepare reports on the status of their work, and to come over here to discuss them with us.

I wish also to thank the American investigators who have presented reports on work which may possibly relate to peripheral areas of the scrapie problem. These efforts are recognized to be more difficult, because the work on which these reports have been prepared is incomplete and continuing. Their justification derives from the result that we are now all able to think about the scrapie problem on the basis of the same information.

In my judgment this common basis for consideration is most important.

I would like to make a division in my remarks at this point. What I have said I felt it very important to say. What I have yet to say you are at liberty to take as one man's opinion. It may have no importance whatever. It is an opinion, and I would like to say it, and you may disregard it to whatever extent you see fit.

Those of you who are charged with the responsibility of re-examining and re-aligning the scrapie control program are in the unenviable position of having to take a course of action based upon less facts than you would like to have to arrive at a conclusion, and wherein mature judgment must serve in the absence of fact, and the appraisal of undefined risks must be attempted.

For example, you have been presented with evidence which tends to indicate that scrapie can be transferred from one animal to another under certain artificial conditions, possibly unnatural conditions. You also have evidence before you which shows that under the ordinary conditions under which sheep husbandry is practiced, transmission from one sheep to another does not occur to a significant extent, if at all.

Those of you who are responsible for the regulatory program must decide on the basis of presently available information, supplemented by your best judgment and experience, whether the slim risk of contagion under the ordinary and usual conditions of sheep husbandry is sufficient to justify continuation of the massive slaughter of sheep, realizing from facts which are before you, that most of the sheep are not, and never would be, affected by scrapie.

You must also decide whether scrapie is, or is likely to become, a disease of sufficient economic importance under ordinary practical conditions to warrant a control program of any kind; and, if so, whether such a program shall involve slaughter along genetic lines, and to what extent.

Toward appraising the overall risk of possibly encouraging scrapie in this country by a relaxation of the present program, it was brought out here this morning that the incidence of scrapie in Great Britain is in the order of 1 percent, even in the most susceptible breeds. Thanks to the able and dedicated investigators whose prodigious efforts will ultimately elucidate the scrapie problem on another day in another year, there will be a more complete set of facts upon which you or your successors can again look at the regulatory aspects of scrapie. Their job will be easier than yours. You have our best wishes.

Thank you.

Dr. Anderson:

Dr. King, would you entertain any questions?

Dr. King:

Bear in mind that these are the words of a layman; that this is not a field of specialization for me, and I am in no sense an expert in it.

[1] Director, American Cheviot Sheep Society, Whitehouse Station, New Jersey.

THE SCRAPIE PROBLEM IN THE UNITED STATES

(Statement by P. L. Linn[1])

Mr. Linn:

I would like to see two of Dr. Hourrigan's slides.

Dr. Anderson:

Dr. Hourrigan, would you return to the rostrum for questions on your presentation?

Mr. Linn:

All I want to do is to establish which of those sheep came before the program, I believe in 1956, when they began to retain the sheep, before that time in the States when we operated in more or less of a loose fashion.

Colony Ben?

Dr. Hourrigan:

The Colony Ben situation occurred in 1952.

Mr. Linn:

Patrick would have been about 1951, 1950, or 1951.

Dr. Hourrigan:

Yes, at least one of them was found to be affected in California in 1952. Those in Illinois were in--

Mr. Linn:

The reason I am running down through this list is to see which of the sheep population, the progeny of these rams, whether it was before they were gathered up. Now at one time we didn't gather them up, and I think that time was about 1956.

Colony Ben I think we are agreed on, and the Patrick ram. The Huron Grove ram would have been debatable. The next one is of Canadian registration; I can't tell about that. I think that one perhaps is also before. Likewise Hudson.

Those Canadian registrations, I can't tell you about.

Those are borderline; right close to the line when the program was changed.

Porteus was probably in 1953; 1952, or 1953.

Dr. Hourrigan:

Badly Advance Guard was the British ram that died after showing signs resembling those of scrapie; however, he was not a confirmed case.

Mr. Linn:

I see. Well if it had National registration on it then we could tell; but some of them never got over here.

The Clarkson ram would be a ram of about 1950, according to the registration number.

Borwick is probably 1953. That would be before the time.

Claspell would be about 1955, along in there; or probably before that date.

Lost Acres would be before.

The next one, Sanderson, is English.

The next one, Floyd L. Edwards, is also English.

McIntyre is English or Canadian. At least we don't have any information on that. There might be an American association there, too. That would be 1950.

Steddom would probably be 1955 or 1956. It's debatable. Likewise for Heggemeier.

Willow Brook would be prior to that time, about 1955.

Van Vleet would be about 1953.

G. B. Vance would be about 1951.

H. Davis would be in the borderline of 1955 or 1956.

Finch would be 1950.

Warwick would be about 1951. And the same for the next Warwick.

[1]President, National Suffolk Sheep Association, Bucyrus, Ohio.

Hursts would be about 1953.

Kane would be about 1953.

Dickes would be about 1953.

The next, Ewing, would be about 1953 or 1954.

Middlemus would be a Cheviot, and Helms was a Cheviot. And the reason I brought that out, of course there are a lot of rams, but these had more than two progeny.

What I wanted to do was get the data before yourself and myself as to how many of those rams were before the time that the present program was adopted, because it has some connection with any analysis of the data.

Dr. Hourrigan:

Thank you.

Question:

I am not at all clear as to what Mr. Linn is trying to bring out.

Mr. Linn:

These charts, these two pages of charts, list rams responsible for two or more cases of scrapie, and all of them put together account for an overwhelming percentage of cases in the United States. As I named them off what I was doing was trying to establish the date of birth of the animal, which will indicate the probable date or time that he was a sire. And I projected that against a change in the program in 1956, because in 1956 there was a radical change in the program, not only with respect to source flocks but also in regard to other parts of the program in which the progeny of these animals from these flocks had been diffused widely, because the program of containment now in use wasn't then in existence. They account for a failure to retain the scrapie potential by the bulk of the rams indicated on the chart which were the most prolific.

Now there is one other question it might explain, another thing which you mentioned, Dr. Hourrigan, and this might interest our Canadian friends. We had a lot of statistical data on the movement of sheep. I should have perhaps made an introduction, but let us say that Tom King did such a nice job that I will just let it go.

But what I was going to say, this may explain some reasons why you find the sheep coming down from Canada.

It happens that I have been President of the National Suffolk Sheep Association for either 5 or 6 years. In fact, I have had the treatment in this deal; and, in fact, I think I am the only one on the Board that has been on for the duration. And then I had a little lapse of about 6 months. So if you get too cruel, please remember that I do have a kind of a tough position to fulfill. And yet I think you find at least I try to be dedicated to the interests of the people I try to represent.

Now I want to make this statement: In all history, in all species of livestock in this country no breed association has ever had the phenomenal growth of the National Suffolk Sheep Association in the particular period that I have been connected with it; and it happens to be this very period that I am talking about.

Now with that we have another association in this country, and during that time--that is, from 1952 until about 1960--it increased its registration from about 8000 annually to about 21,000. At the same period of time we increased from 8000 to about 24,000. Now since that time we have maintained 22,000; and then I think the other Association this last year had about 10,000; so the total Suffolk sheep registration in this country is approximately 32,000 annually. The combined total of the two is the greatest of any breed.

Now when you consider that phenomenal growth in the very particular period that we are talking about, naturally a lot of that number of sheep came down from Canada. So we got it from Canada. And Canada got it from England. So it was only natural that this would happen. And I don't think we ought to read too much into it; because a lot of those animals that I saw, particularly on the latter data, were animals that were strictly in this country. But that doesn't mean anything; because, after all, they had their parenthood back from sheep that had come from Canada, and from Canada they came over here.

Well naturally I don't believe Suffolks were in our country to any great extent more than 20 years ago.

So I just wanted to give that briefly. I am not trying to give any statistical data. I just think it is interesting to note, and I think it goes some way toward explaining the situation.

I want to say this about Michigan, which might be of interest to you. The breeders in

this country knew--at least some breeders in the country knew of the Michigan situation as far as perhaps 5 years or 6 years before. Many of them didn't even know what they were dealing with, and perhaps some of them did. But at that time, even up to 1953, the regulatory officials were not too much concerned about what happened with scrapie in this country. So if there is any criticism of anybody--let's not criticize anybody, as far as I'm concerned; let's just face the few facts of what took place.

Now how much of it was before 1946 I don't know. But I do know that sheep breeders associated with the Michigan flock have told me reliably that that is the case.

I am not going to make any comment on what has been happening here in the last 3 1/2 days, because I don't want to get into any arguments.

So I am going to proceed to present what I have put down here, and if I am a little pointed--I am a little bit in the place that Tom King was when you questioned him on what he said before the House Committee on H.R. 4217. In other words, Tom King wrote an item--I was there when he wrote it. It was thrown together very quickly. We were limited, I think, to an hour's time, and they finally gave us more. And if anybody can say as much as Tom King was pressed to say at that time, in that short a space, and not have someplace for somebody to get ahold of something, he certainly is better than I. Because I think Tom has done a marvelous job when you look it over.

Now one thing that I would like to at least throw thought upon is this matter of an objective analysis of what the economic significance is. Now to do that--and this casts no reflection on the people I am going to quote; because I think the best place to do that is to go back to England, because there they have had it for 250 years and you can get some idea.

So I would just like to throw this information out for the simple reason that we have sat up here for 3 1/2 days and we have seen charts involving 65 percent scrapie, and we never did see one where they didn't have scrapie; in fact, everyone of them had a lot of scrapie. So we get to the point where scrapie is just crawling everywhere around.

But they are rather respectable people, and I think things happen over there a good bit like they do over here.

First I would like to go back here, before things begin to get hot and we get maybe a little different viewpoint.

This happens to be one of your papers, Dr. Stamp, and part of what you said was similar to it. And I want to read just the first paragraph to give your viewpoint. And this was in 1957. This is what Dr. Stamp said:

"Scrapie disease of sheep has been present in Great Britain for very many years. Over this long time it has not given rise to notable losses among our flocks as a whole. But from time to time it has presented a serious problem to individual flock masters. Perhaps its more important effect is the interference with the export trade in the pedigree sheep. It is not only causing considerable financial loss to individual pedigree sheep breeders in Britain, but also a considerable loss to our national prestige; since we pride ourselves on being unrivaled in the breeding of good stock."

And then if I may shorten this a little bit, because he mentioned this and I want to be fair to him, he also stated the other day--and I think this is verbatim--that "... as far as commercial sheep it is of very little significance."

And at the same time being fair on the other side, he said occasionally there is a sheep breeder who has serious losses.

Now I will treat our other English friend, who said in 1959, in San Francisco--Dr. Gordon--This is quoted from him. He said:

"This disease has been known in Western Europe for over two centuries where it has waxed and waned in different breeds over long periods of years. While the incidence of infection is not unusually high, the disease can sometimes cause devastating loss as in flocks of purebred sheep; but it is not usually the cause of serious loss in commercial flocks."

Now I don't happen to have a paper to quote from Mr. Parry, but his position is about the same thing.

All I mention this for is to get a real picture for what you really have over there. I have not been over there; but I trust that you fellows in 1957 were telling the truth.

There is one other thing, which is irrelevant to what I was going to say; but you have one statement in here that I hope you people will consider, because I have had experience with

this one. And that is this particular statement where you said that any indemnity scheme would be quite expensive, but that was not the main point. That was unless you have a system of diagnosis you are liable to have a lot of acrimony--"endless confusion and acrimony."

Now I know that, because I am in the saddle, and I know what you get out of that kind of a situation, because everybody is at everyone else's throat.

Now I have a brief statement to read and it makes a few points; and I hope, in trying to make it brief--I have an incapacity: it's hard for me to be heard, so I have a tendency to talk sharp and a little harshly, so don't take offense at it. I simply want to cover as much territory as I can, and I will be brief.

What I want to say is, and it's not too far from what I think Dr. Wells said: What research might develop does not have an important bearing on whether there is justification for the present program. Research is good. And I don't want to be misunderstood now. The reason for this is very simple. The sole and only condition for justification of this program rests on one thing namely, and that is the degree of success you have in tracing the movement, and the destruction of animals which are supposed to be destroyed.

Now the only people who have that information are the people who keep the records. And in this case that happens to be us, partly. There are some others who have it too, but we happened to be one of them.

And you are concerned with movement of the sheep affected; mostly it is the sheep that are affected.

On the basis of our records these are some points to consider. And I mentioned this partly here a moment ago:

No. 1, the movement of sheep from the first--that's about fifty--flocks were virtually not traced, gathered, or destroyed at all.

No. 2, the movement, the gathering, or the destruction of a much larger group, the commercial sheep, has, is, and will continue to be virtually ignored under the present system. The only way to eliminate this latter instance is for the commercial sheep organizations, such as the National Wool Growers, the Ohio Wool Growers, the California Wool Growers, and so on, to provide for such recording of the individual movements of each animal.

Unfortunately, unlike foot and mouth disease, where movement of animals is a consideration of only several months or several weeks, the scrapie program involves movement of animals in a multitude of places for up to 3 1/2 years, which I would consider an impossibility per se. No breed association has the economic capacity to provide for such situations for long periods of time. We are not cognizant of any move to provide this service of similar character as required of the purebred associations by the sheep organizations representing commercial interests.

No. 3, this point of information I am about to bring up--as I say, I am not going to argue with anybody about it. It is information we have. It involves people in our organization, and I won't reveal it. But you can be sure that it is authentic and has been established between the Secretary and myself, who are the only people I know that have it. And we didn't do it on a large scale because it is simply again that we don't have the capacity to do these things on a large scale. In fact, at the present moment I have fifteen letters or so lying on my desk, plus those liable to come in from Missouri, to try to unravel and satisfy and keep peace within the family, for the last case, the big case, the Heggemeier case. It's impossible. I haven't caught up. And I have lambs at home dying now, and I should be home.

But this is what I want to say. We don't guess about this: we know because we have records, on this one particular case at least, and I am sure others will come up too--and I think there might be others that are larger in percentage, and there might be a lot of others that are a lot less, I'm quite sure of that:

But we know that sometimes as little as 10 to 20 percent of the animals which are supposed to be destroyed are actually destroyed; and that is assuming that everybody is honest.

We also know that it is not too important that the remaining 80 or 90 percent are not destroyed, because nothing seems to happen anyway in the way of scrapie in the subsequent 3 1/2 years or more, on the basis of the records of the cases of incidence of scrapie throughout America.

Do you understand what I mean?

If you go back to those sheep in this particular case that have been traced back and

checked against the cases of scrapie developed in this country--now that doesn't mean that a lot of it wasn't underground: I won't say that, because I don't know, but there has been none reported in that flock. And making a quick analysis of those flocks where even the incidence is high, for some strange reason nothing happened once it's there. I think there were a few scrapie cases. I didn't follow your figures too much because, after all, I was trying to worry about myself a little in preparing this statement.

All right. No. 4: In addition to these things we must also consider the consideration pointed out by Dr. Stamp that 10 percent of the scrapie cases--I hope I'm right on this--that were diagnosed post-mortem never showed up in the field, because of the fact that they died before the scrapie manifested itself. That's 10 percent.

And then this next one is also probably developed from Moredun, and I wanted to bring this in together with a few things I picked up, these are all new to me--and that is that they had a few cases where an animal died quickly without very much chance for anybody to diagnose it as to what it had, and it turned out, according to your laboratory findings, to be scrapie.

Now I add those to it.

And then finally--this is more of a scientific nature, and it happens to be a veterinarian, so, if you want to quarrel with anybody, quarrel with him. And I refer particularly to the Evaluation Committee--and I have a few of these articles with me, and if anybody wants them I will give them to you. They are out of a magazine. But I want to refer to certain paragraphs. They are from THE SHEPHERD, January, 1964--and I call this Point No. 5, and I am going to read from it, from Dr. Beck's article, "Scrapie." And Dr. Beck is from Michigan State University, and I want to read this because you veterinary people would probably be most interested.

"Following the USDA program, 60,952 sheep have been slaughtered prior to 1963. Are we justified in slaughtering this number of sheep just because there had been an association of some kind with the 200 actual cases, in the light of the fact that contact transmission under field conditions has never truly been proven to occur to the satisfaction of scientific research workers?

"If we follow the genetic theory, then only individuals genetically related to these sheep should be sacrificed."

"If we follow the infection theory, then there might be some justification for slaughtering contact individuals; but with the very low and often non-existent incidence of subsequent infection in quarantine flocks, it raises a serious question. The Federal program further follows up animals that originated from the flock in which some infection has been found. These animals are also destroyed."

Now I want to depart here just a minute. Dr. Beck hasn't included the source flocks, which is even longer.

And then quoting again:

"If we follow the infection theory on these individuals, then the entire flocks in which they are located might also be considered to be exposed. This situation becomes a snowballing threat to the entire sheep population."

My only purpose in bringing this up was to show this potential of scrapie exposure, and so forth, that has been taking place over the years. And this would be true whether scrapie was hereditary or caused by some unknown infection.

It is quite important, therefore, that any scrapie program be designed so as to attract the greatest possible support and cooperation from the people really affected by such a program. The object, indeed, should be to have the program acceptable to those who are having their animals destroyed.

Thank you.

DISCUSSION

Dr. Anderson:

Mr. Linn, I believe you mentioned that the scrapie program has resulted in a tremendous economic loss. And I agree that some have suffered severe losses. But I note here that during a 10- or 12-year period that your association membership has increased from 8,000 to 22,000, or about 14,000 increase. Has this interfered with the demand for, or the popularity of the breed?

Mr. Linn:

Well that's a pretty hard statement to make. I suppose there is a lot of "conflict of influences." There may be traffic in lower-grade

animals that this might even encourage. I don't know.

The growth of the Suffolk has been phenomenal, and part of the reason for that is that during the war we made a sudden about-face in the production of animals. We wanted something that would produce meat fast instead of a short, light individual. That was the philosophy in regard to cattle and everything else, and the Suffolks came in with that.

Now I would say that this [the scrapie program] could hurt some of the others more than it would us, because of the size. If you applied the loss that we had against the Cheviots, for example, with one-fifth the registration, you would practically wipe out the breed. But the growth is so phenomenal, when you consider it against 40,000, that it probably has not caused much damage. I can see where it could undermine not only the Suffolks, but we are in a period when the whole sheep industry is being affected by the philosophy that the product is too fat, too expensive.

I don't know whether that answers your question. It's the type of question that you can't answer specifically.

Dr. Stuart:

This change in 1956 of the program that you referred to: I would like to have you refresh my memory on what that change was, just what it consisted of.

Mr. Linn:

That took in the source flocks. Also at that time they began to contain the animals more.

Dr. Stuart:

Dr. Hourrigan, was there any change made in the California program in 1956 other than taking the source flocks?

Dr. Hourrigan:

The program in California began with taking the infected flocks, and exposed sheep moved from the flocks, and the immediate progeny of the exposed sheep. At that time, source flocks were not involved in California but were in Canada. These are the procedures which the program essentially follows now.

However, at that time the program in Ohio only included slaughtering the infected flock. The program was broadened in 1957 to include source flocks as well as infected flocks. It also included exposed sheep moved from both categories, and their immediate progeny.

Dr. Stuart:

But it remained the same in California, with the exception of the source flocks?

Dr. Hourrigan:

Yes, that is correct.

Dr. Stuart:

I think you are aware of the fact that last summer California went through a 42-month period without the appearance of the disease. In other words, we felt that we had completely eradicated scrapie. There had been no change made in the program other than we did include source flocks. And I would like to convey that information to Dr. Wells.

It is generally known that we felt that we did eradicate scrapie. I am convinced that we eradicated scrapie in the State of California using the present program.

Mr. Linn:

Dr. Stuart, with regard to Ohio, at that particular time you are talking about, we actually had a flock in quarantine in the State of Ohio for better than a year. And, foolishly, the owner didn't breed his sheep. I shouldn't say "foolishly": he just saved the taxpayers some money. And this kept on for about 12 months. And, incidentally, we had a flock in Illinois in quarantine for about 14 months. That owner was finally paid, but the one in Ohio, poor guy: he couldn't afford it, but he was tied up for about 12 or 15 months, and finally ended up with a big struggle through the Sundry Claims Board. I happened to be one of the appraisers. He nearly didn't get his money; except that when I appraised the sheep, it [the appraisal] was notarized, and therefore everything worked out.

So, these things get far-fetched.

Our trouble is that in our Association we are [active] in fifty States, and each one of these operates independently.

Mr. Yohe:

With regard to the sheep that have actually been slaughtered under the program, how many are there?

Dr. Hourrigan:

The figure is approximately 80,000 sheep.

Mr. Yohe:

If I interpret the graph concerning the number of infected flocks per year correctly, even though we had some rather substantial peaks and downs, actually the curve is sloping upward. So that the general long-term trend is to have more flocks, not less, over this period of time.

Dr. Hourrigan:

There was a peak in fiscal year 1956 when there were 23 infected flocks. Since then the number of outbreaks has dropped from that figure but remains in the 7-14 area.

Mr. Yohe:

I think if you drew a mid-line through there you would actually have it going up. This leads me to the general conclusion that we are not quite holding our own on this, program-wise.

And then there is another question I would like to ask, coming back to the Suffolks.

Most of these outbreaks involved Suffolks, and apparently we have increased our population of Suffolks over this time by perhaps even in the nature of four of five times. Actually, don't you feel, then, Mr. Linn, that our percentage of scrapie in Suffolks has probably gone down?

Mr. Linn:

I will have to ask what years you are referring to. I will give you the extremes on Suffolk registration. In other words, the American was up to 21,000 in 1960, and we hit our peak then, about 24,000--that gives us about 45,000. But at the present moment we are back to 32,000, so there is quite a variance there.

In about 1952, the total Suffolk registration would have been roughly about 17,000. And then they progressively increased. The American was quite erratic. In one year it went up 10,000 and the next down 10,000, and that was right about in the middle of that period. So it is pretty hard to tell.

Also, suppose there was an outbreak of scrapie in one of the Western flocks, and they went out and raised a lot of sheep, but perhaps were delayed in registering them for some time: in view of the numbers involved here, it would be considerable, and would affect your chart considerably.

Mr. Yohe:

Well, at any rate, perhaps this four times is far too many. Perhaps it should be double, or something like that.

I realize that what I am asking you is perhaps not a fair question. But this is more for your opinion than for facts.

Do you feel that the incidence of scrapie in Suffolks has gone down, or held its own?

Mr. Linn:

I don't see much change, based on figures. It's erratic in the figures. But when you figure the 17,000 in 1952, and by 1954, we probably had as high between the two associations as 25,000 or 26,000. So you see as far as those figures are concerned it is not sufficiently significant, I would say.

Dr. Hourrigan:

I think, Mr. Yohe, in further answer to your question, that we might point out that in 1957, when we had the peak of outbreaks, it was obvious that if something wasn't done, source flocks would continue to disseminate scrapie, causing additional outbreaks; it was with that background that the program was changed at that point.

Mr. Linn:

I think, Dr. Hourrigan, you agree with me that charts are a risky business in the situation in the United States; because, just like those movements, they don't mean too darn much. Because if you have one or two affected, and the numbers are so small, they radically affect your charts.

Mr. Yohe:

With the number of sheep involved, 200 sheep, would be hard to chart, I suppose.

Dr. Hourrigan:

As Mr. Linn says, the size of the infected flock makes quite a difference. One infected flock in California had 7,000 sheep, I think, Dr. Stuart. This, of course, increased the number of sheep slaughtered appreciably.

In 1953, 1,352 sheep were slaughtered; in 1954, 273 sheep, in 1955, 3,761 in 1956, 5,116;

in 1957, 16,838; 1958, 4,799; 1959, 7,029; 1960, 10,543; 1961, 2,777; 1962, 8,464; 1963, 5,079; and in 1964, 5,292.

Mr. Yohe:

One of the things I think that concerns us all is--and you are the one who can answer this best--What is this doing to the Suffolk breed? Are we, in essence, by this program, eliminating your very best bloodliness? In other words, have we reached the point where the Suffolk breed is actually being severely handicapped? I am not talking in numbers: the numbers apparently have increased but so far as the top animals are concerned?

Mr. Linn:

I would think--and I think this is borne out by the shows that we have in various places: it happens in Ohio State, which has one of the greatest shows--they call it "The Greatest Show on Earth"--it's advertised as that, and everybody pretty well accepts it. The International at Chicago is a great show. Another great show is held in Illinois. And when you see these shows, of course, all the good animals are there because, you have to remember, the people make their business there, and they aim to have the best. The quality of the sheep being presented [is going down], and the depth in the line is becoming less and less, as far as quality is concerned. Quantitatively, I would say that the sheep population [is not affected]. The figure I gave you is not accepted in the West, and I can't quite calculate that figure because, you've got to remember, in the West you have a lot of sheep that aren't registered. And if you have a case of scrapie and a lot of the sheep get registered, they bring them in, and then you get a distorted picture.

I just wanted to mention that; because, in the first place, if you are affected by a case there, and you bring them in quantitatively, you are basing that larger group of sheep against 10,000. Whereas, we have smaller flocks, perhaps half as large, and if we get a case of scrapie on the average we come in there and project them against our 22,000. So you get a distorted picture.

But, on answer to your question, I would say definitely the quality, it appears to us, is going down. But quantitatively it [the sheep population] isn't affected.

Now I think--this is my own position--that so far as a lot of the other breeds are concerned--and I don't know whether this applies to Cheviot people or not, but I think the Cheviot people [could be included], one or two more instances could hurt them very badly, because they are only working with four or five thousand sheep. And that's the story in a good many breeds.

I think that's what has happened; most significant of all, and the most harmful of all, is the lack of confidence being built in the purebred industry.

Mr. Gates:

I would just like to point out, without drawing any conclusions, that [in connection with] the Cheviots, being a numerically small breed in this country, if we draft the number of sheep registered in a line comparable to the incidence of scrapie in the breed, we find a 5-percent reduction in registration numbers since this [scrapie] has hit our breed. I would like to point out also that we were very unfortunate in that scrapie hit one of our most diversified breeders.

But I do think that the Evaluation Group should know that this program has had, [a suppressing effect]. Without making an analysis, with the advent of this program the number of Cheviot sheep in this country has decreased--we were going up 4 and 5 percent per year in registration number until approximately 1954, 1955, and 1956. And since that time we have started coming down.

So, with a small breed, we think this program has had a very suppressive effect on our registration numbers, and, of course, has created apprehension among the buyers.

Question:

On the graphs showing the incidence of scrapie with the high point in 1956, I would like to ask Dr. Hourrigan, does he think that as the information is fed out to the various farmers, they are liable to go underground and not report any scrapie sheep?

Dr. Hourrigan:

I don't know. That is a possibility, of course.

In trying to assess this matter of scrapie, or any other disease, going underground, I am sure there is always that possibility.

Various factors impinge upon it and make it more likely or less likely. I feel that in most cases where owners had scrapie and didn't report it, they honestly didn't know they had scrapie. Now perhaps some of them did. I am sure there is the odd one that did. But I think most of them didn't.

Mr. Linn:

I would like to make a little comment on that; after all, when you live with these people, and you try to iron them out and keep everybody from one another's throat, you get really close to them.

But this touches me pretty deeply, and it irritates me a little bit, and I mean no offense by it.

But Dr. Dickinson was talking, I believe, about a case where he was alarmed because some English shepherd over there was going to begin to hide his sheep if he lost the value of the darn carcass. In other words, even the carcass value is an important consideration, if you create a condition where you can lose the carcass value.

But we have people here who lose 25 or 50 thousand dollars--a lifetime's effort, prestige. And you run that danger, I think.

Dr. King:

I have one small question of Dr. Hourrigan.

Early in his presentation he referred to the California case, which, I believe, was observed early in the scrapie program. There were 21 cases either diagnosed clinically as scrapie, or laboratory confirmed.

My question is: Is my understanding correct that those 21 sheep came in from an outside flock?

Dr. Hourrigan:

Dr. King, all of those sheep from that particular ram--if you will recall those lines radiating down--all of those were the immediate progeny of ewes purchased from the source flock in Canada, the ewes having been bred in the source flock and purchased as bred ews. Althogether, of the 21 affected sheep, 13 were born in the two California flocks concerned, and eight had been introduced from other flocks.

Dr. King:

They came from a common source?

Dr. Hourrigan:

Seven of the affected animals were the immediate progeny of ewes bred to Canadian ram, Colony Ben, before the ewes left Canada. The other 14 affected sheep were by ten different rams, some of which were from Canada or Britain and some were American-born. Each of the 21 affected sheep was dropped by a different ewe.

I have a graph showing the average selling price of rams of different breeds sold over the last 10 years at the National Ram Sale. I don't know whether it is appropriate to show it here or not?

Dr. Anderson:

I believe it is, if we are talking about the economic effect and the impact of the program on the industry.

May I ask Mr. Linn a question? I don't want to be facetious in this, but you referred to what appeared to be a lowering of the quality in the Suffolk breed in recent years. I heard a gentleman at noon make the remark that the breeders felt that they needed imported British sheep to maintain the quality of the sheep in this country.

Mr. Linn:

And you want me to comment on that? All right, I'll comment on that.

But first, I wonder how come I said that, how bad the quality of sheep was getting in the United States, because it just happens that I won the International myself. And I've got myself in a hole here, having won the International, telling you that the quality has gone down.

But let me hedge and say that the depth wasn't very deep.

There is a strange thing about this, and I always have some feeling if anybody questions the quality of the English shepherd. It is not only the English shepherd--some of my Scottish friends in Canada have pointed this out to me. It is not only the English shepherd and the improvement of sheep, but in every field of livestock-breeding throughout history. Now here are the little British Isles, no larger than one of our States, or like Ohio and Indiana put together, and for some reason or other they are such geniuses--if you want to call them that--at the production of purebred,

pedigreed livestock that we have to turn to them from time to time to get the basic good stock. I have no quarrel about it. I am willing to accept that hypothesis without question.

Does that answer pretty well the question?

Now that is what is particularly needed in Canada, if I may refer to Canada. I do know the situation.

But take the North Country Cheviot. They embroiled in a Western affair this year, or in the last year and a half, in which there was a heavy concentration of North Country Cheviots. But there weren't many North Country Cheviots in the North Amercian continent anyway, because they were only brought over here in the last few years--or, I should say, not very many years ago. They took a terrific beating.

Now there is one comment I'd like to make, if I may, and that is with regard to the total number of sheep slaughtered in this country under the scrapie eradication program. The last figure that I looked up in the hearings that you provided was around 60,500 sheep. And I think we can agree that there are probably four or five thousand more, counting the Illinois situation.

Dr. Hourrigan:

Altogether, Mr. Linn, through fiscal year 1963 it was 66,000 sheep.

Mr. Linn:

And then these in Illinois have been slaughtered since then, haven't they?

Dr. Hourrigan:

Most of those involved in the Illinois outbreak, yes. Figure No. 7 shows the average price in dollars of rams of various breeds sold at the National Ram Sale through the years 1950 to 1963. There have been fluctuations. By and large, I think one breed has pretty much maintained itself among others as far as overall prices are concerned. Mr. Timm might have a better idea than I as to prices.

The double line represents Suffolk rams. The Suffolks have maintained their relative price quite well during the eradication program and have, in fact, from a comparative standpoint within other breeds, gained rather than lost.

Mr. Linn:

I would think that the sales in the East probably wouldn't appear quite that favorably. Some of these other people may have had experience with that, but I don't believe they appear quite that favorable.

I have one more comment in regard to Dr. Stuart's remarks. I would like to answer his question.

From the standpoint of our Association--in other words, I have been hearing something about scrapie all week. And you know, we are involved heavily, and the repercussions from the public are great. The situation is difficult, and it is very erratic. Some States don't have any problems, but Illinois is a particular one. I want to show you the size of one of the things. Take the problem in Illinois for the last two or three, or four years. I believe the total figure is around 6,000 sheep killed, mostly purebreds, involving about $474,000, State and Federal. That's the State of Illinois alone, in a 4-year period (1961 - 1964). Sheep slaughtered in Illinois since 1960 are as follows: 1960, 1,882; 1961, 1,056; 1962, 1,848; 1963, 775; and 1964, 2,236.

Dr. Mulhern:

The point comes up from time to time on the disease going underground. And being realistic in dealing with programs, this generally is a part of any kind of a program. But fortunately in the past, according to our record of eradication, we have been able, somehow, to overcome this, or we wouldn't reach the final goal.

But since this is vital, and particularly with this type of disease, it is a very serious problem. The fact that it may be going underground may show the ultimate goal of eradication quite difficult to achieve, and leaves us only with the conclusion that the disease will spread to all breeds through this type of thing. That is the danger of it.

Certainly this is an area of concern. In order to try to eliminate this disease, regardless of where it may be in the breeds, because of the seriousness of the problem, we should bring constructive comments into this area as to what can be done to alleviate the difficulty.

I think we are all in here, feeling that the desired goal is to eliminate scrapie completely from our sheep flocks. Now, how we can do

this is the key that I think would be most constructive; to see that as a result of the disease going underground, scrapie will spread. It isn't that the program fails, it's the fact that the disease is disseminated through all flocks.

Mr. Linn:

Well, Dr. Mulhern, if I may comment on that a little bit--and again, I am not a veterinarian, but I do want to point out one thing. I stayed away from this [problem of the disease going underground] because I didn't want to start anything along that line. But in connection with the question, you remember that I had five different points, one of which Dr. Beck brought up with regard to these flocks: if we take out 5 head or 1 head, and then that exposes all the rest, (and again I will have to depend on someone else who has a mathematical brain) we have the possibility, over this period of 10 or 12 years, or 15, of let's say millions or trillions of exposures, but the disease didn't spread to any other breed, so it can't be too highly contagious.

I'm not trying to prove anything, but I'm just trying to point out that those multi-billions or trillions of exposures did take place, and the disease didn't spread to any other breed in this country.

Now I have one more thing, but I don't want to monopolize the time. There are some other breeders here, and somebody else wants to talk.

Dr. Gordon:

Mr. Chairman, there is one remark made by Mr. Linn that I would like to modify.

In the course of your earlier remarks, Mr. Linn, you said that the records of the United States Department of Agriculture on scrapie in this country were unreliable. I think you probably meant that they were unrealistic in relation to the true incidence of scrapie.

Mr. Linn:

I'm sorry if I used that word. I probably should have said "unrealistic," or perhaps too small a number. They weren't quite as good as ours, and ours weren't good enough.

Dr. Gordon:

I am very pleased that you agree with this and made that amendment, because I think the records of the United States Department of Agriculture about scrapie are very good.

Dr. Anderson:

Dr. Wells made a statement a while ago that I would like to elicit some comments on from Dr. Gordon, Dr. Stamp, or Dr. Hourrigan.

Dr. Wells, I understood you to say that one of the problems we were facing in our program was that we do not have a diagnostic test for scrapie. I was under the impression, in following that through, that that would mean that we are unable to recognize scrapie; that we have no means of determining that scrapie exists in an animal, or that an animal is affected with scrapie.

If you are inferring that we do not have serological tests, that is one thing. But saying that we do not have a testing device to determine if the animal is affected with scrapie--I seem to be at a loss.

Dr. Wells:

I think what I said, Mr. Chairman, was that one of our problems is that we do not have a diagnostic test to indicate the presence of the disease prior to the onset of clinical evidence.

Dr. Anderson:

Fine. I misunderstood that last point. Is there any other sheepman or breeder, or anyone else, here who would like to comment on the program for this country, or the disease? Are there any other questions that you want to direct to the research workers, or others who are not members of our group? If not, this phase of our evaluation meeting will come to a close. And I want to thank all of you for attending and participating. It will be very helpful to us in our deliberations on the disease, and the cooperative program that is being followed.

Thank you very much.

(Whereupon, at 4:00 p.m., January 30, 1964, the Scrapie Seminar was concluded.)

☆ U.S. GOVERNMENT PRINTING OFFICE : 1966 O—216-471